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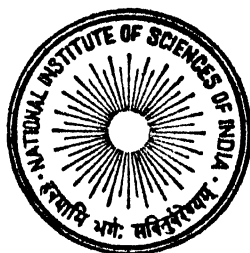
PART B

BIOLOGICAL SCIENCES

No 1

26 February 1956

Vol 22



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NEW DELHI

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PROCEEDINGS OF THE NATIONAL INSTITUTE OF SCIENCES OF INDIA

No 1

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Vol 22

CONTENTS

Analysis of Chromosomes in Two Genera of Microhylidae (Amphibia: Anura) by A. R. KASTURI BAI, M.Sc., D.Phil.	1
Morphology of <i>Pallavicinia</i> with reference to its Species Problem and the Indi- viduality of <i>Pallaviciniaceae</i> by P. KACHROO, M.Sc., Ph.D., F.L.S.	6
The Heart and its Conducting System in the common Indian Fowl by RAVI PRAKASH, M.Sc., Ph.D.	22
Morphology and Histology of the Air-bladder of <i>Hilsa ilisha</i> (Hamilton) and <i>Gadusia chapra</i> (Hamilton) and their connection with the Internal Ear by P. N. SRIVASTAVA, M.Sc., D.Phil.	28
On the Morphogenesis of the Yolk-sac Gland in Chiroptera by SINDHU MOGHE	34
Some Observations on the Foetal Membranes of the Indian Palmcivet, <i>Paradoxurus</i> <i>hermaphroditus hermaphroditus</i> (Schrater) by M. A. MOGHE, M.A., M.Sc., Ph.D., F.Z.S., F.N.I., F.A.Sc.	41
On the Development and Placentation of a Meghachiropteran Bat— <i>Cynopterus</i> <i>sphinx gangeticus</i> (Anderson) by M. A. MOGHE	48

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No 2

26 April 1956

Vol 22

CONTENTS

An Investigation on the Effect of certain Chemicals on the Nucleus, and their Possibilities in Chromosome Analysis in Plants by A. K. SHARMA, D.Sc., and A. K. BAL, M.Sc.	57
A Morphological and Cytological Study of the Gametogenesis and Oospore Formation in <i>Albugo</i> Species on <i>Ipomoea hederacea</i> by K. M. SAFERULLA, M.Sc., Ph.D., and M. J. THIRUMALACHAR, D.Sc., Ph.D.	69
Microflora and Age of Punjab Saline Series from Dhariala Well No. 1, Salt Range, West Pakistan by A. BOSE, M.Sc.	77
On a New Species of <i>Platycephalus</i> by L. F. DE BEAUFORT, Ph.D.	83
Studies on the Development of the Embryo of <i>Oryza sativa</i> L. and the Homologies of its Parts by B. SAHA, M.Sc.	86
Studies on the Physiology of Rice. XI. Vernalization and Devernalization of Winter and Summer Varieties by S. M. SIRCAR, M.Sc., Ph.D., D.I.C., F.N.I., and MISS ARATI ROY, M.Sc.	102

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**PROCEEDINGS OF
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No 3

26 June 1956

Vol 22

CONTENTS

Studies on Cytochemistry of Hormone Action. Part XVI. Further Evidence of the Enhancement of Androgenic Action of Testosterone Propionate by Progesterone <i>by</i> A. B. KAR, M.Sc., Ph.D. <i>and</i> N. N. DE, M.B.B.S.	..	113
The Incidence and Heredity of Haemophilia in India <i>by</i> S. S. SARKAR, D.Sc.	..	117
Studies on the Pollution of the Bhadra River Fisheries at Bhadravathi (Mysore State) with Industrial Effluents <i>by</i> A. DAVID	132

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No 4

26 August 1956

Vol 22

CONTENTS

Studies on the Neurosecretory System of <i>Iphita limbata</i> Stål. (Pyrrhocoridae : Hemiptera). Part IV. Observations on the Structure and Functions of the Corpora Cardiaca of the Adult Insect by K. K. NAYAR, M.A., Ph.D.	171
Classification of the Vegetation of India, Pakistan and Burma according to Effective Precipitation by F. R. BHARUCHA, M.Sc., D.Sc., F.N.I. and G. Y. SHANBHAG, M.Sc., Ph.D.	185
Studies on Cellulolytic Activity of Fusaria with reference to Bacterial and other Cellulose Substrates by C. S. VENKATA RAM	204
Studies on the Physiology of Rice. XII. Culture of excised Embryos in relation to Endosperm Auxin and other Growth Factors by S. M. SIRCAR, M.Sc., Ph.D., D.I.C., F.N.I. and A. N. LAHIRI, M.Sc.	212

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PROCEEDINGS OF
THE NATIONAL INSTITUTE OF SCIENCES OF INDIA

No 5

26 October 1956

Vol 22

CONTENTS

Studies in the Amino Acid Composition of <i>Fusarium</i> Mycelium by C. S. VENKATA RAM	227
Nodal Anatomy and the Vascular System of the Shoot of Rice Plant (<i>Oryza sativa</i> L.) by G. P. MAJUMDAR, M.Sc., Ph.D., F.N.I., and B. SAHA, M.Sc. ..	236
Chromosome Studies in some Indian Barley. I by A. K. SHARMA, D.Sc. ..	246
The Atrioventricular Bundle in the Heart of the Banded Krait, <i>Bungarus fasciatus</i> by RAVI PRAKASH, M.Sc., Ph.D., D.Sc., F.A.Z.	255
Studies on the Biology of the Indian Fresh-water Eel, <i>Anguilla bengalensis</i> Gray by V. R. PANTULU, M.A., M.Sc.	259
Behaviour of the Rock Bees, <i>Apis dorsata</i> Fabr., during a Partial Solar Eclipse in India by M. L. ROONWAL, M.Sc., Ph.D., F.N.I.	281

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No 6

26 December 1956

Vol 22

CONTENTS

Sunder Lal Hora (1896-1955). Obituary Notice by M. L. ROONWAL, Ph.D., F.N.I.	287
Life-history of <i>Barbula indica</i> Bridel. by I. BANERJI, D.Sc., F.N.I. and SUBIR SEN	304
On the Biology of <i>Auricularia auricula-Judae</i> (Linn.) Schroet. causing Rot in Elder (<i>Sambucus nigra</i> L.) by SACHINDRANATH BANERJEE, M.Sc., Ph.D. ..	317
Development of the Skull in Catfishes. Part I. Development of the Chondro- cranium in <i>Silonia</i> , <i>Pangasius</i> and <i>Ailia</i> (Schilbeidae) by H. R. SRINIVASACHAR	335
The Occurrence and Activities of certain Bacterial Groups in Off-shore Marine Environments of Gulf of Mannar, off Tuticorin by R. VENKATARAMAN, M.Sc., A.R.I.C. and A. SREENIVASAN, B.Sc.	357

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ANALYSIS OF CHROMOSOMES IN TWO GENERA OF MICROHYLIDAE (AMPHIBIA: ANURA)

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INTRODUCTION

Microhylidae are closely related to Phrynomeridae and they have retained a few primitive features which have been lost by the Ranidae. Members of this family are cryptozoic and myrmecophagous in habit and their distribution ranges from Ceylon, Southern and Eastern India to South-East United States and Rio de la Plata (Parker, 1934). The genus *Microhyla* is common to both Asia and America. In spite of the fairly wide distribution of members of this family, the chromosome numbers of only two genera are known. Makino (1951) cites only one in his Atlas, i.e. *Cacopoides tornieri* (Sato, 1936). More recently, Gowda (1948) has described the chromosomes of *Uperodon systoma*. Two closely allied forms, *Ramanella variegata* Stoliczka and *Microhyla rubra* Jerdon, were chosen for study as they were easily available in Bangalore.

MATERIAL AND METHODS

Specimens were collected throughout the year. The testes in both species are situated in the abdominal cavity attached to the anterior region of the kidney by peritoneal folds. They are small, white, ovoid bodies 2-3 mm. in length and are larger in size during the breeding season than in the dry months.

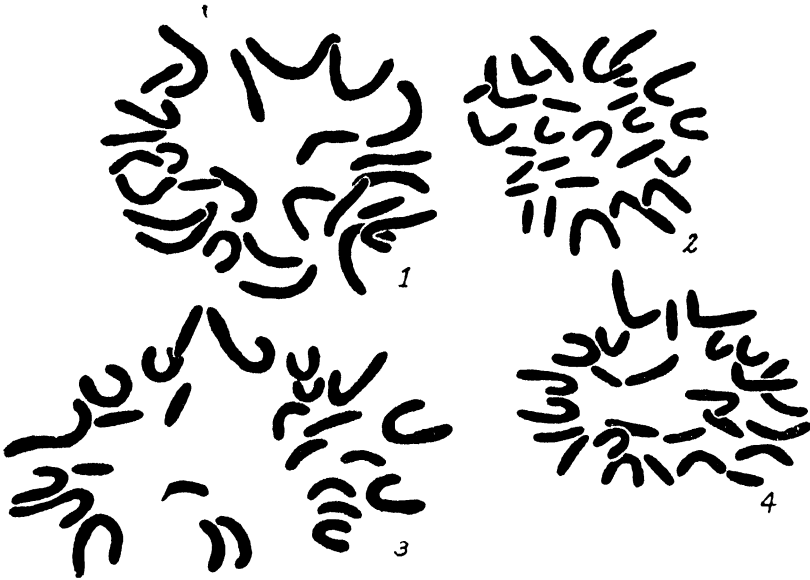
The testes were fixed in Bouin's fluid, Carnoy's fluid and Flemming with acetic acid. Paraffin sections were cut 10 μ in thickness and stained in Heidenhain's haematoxylin but squashes gave better results. Testes fixed in Carnoy for twenty minutes were hydrolysed in normal hydrochloric acid for 10 minutes at 60°C. and stained in Feulgen's leucobasic fuchsin for one hour and squashes were made. Aceto-orcein squashes were also made. Observations were made using 90 \times and 120 \times Zeiss oil immersion objectives and 10 \times and 20 \times eyepieces. The lengths of the chromosomes were measured on camera lucida drawings made at a magnification of 5,700. They were reduced to half their size in reproduction. The analysis was carried out on the same basis as that by Tobias on the albino rat (1947) and *Tatera brantsii* (1952).

OBSERVATIONS

The diploid number of chromosomes as determined in spermatogonial metaphase plates in both *Ramanella* and *Microhyla* is 26. In a number of spermatogonial prometaphase and metaphase nuclei, 26 chromosomes have been counted in both genera. There are 13 pairs of homologous chromosomes and the number was confirmed by counting 13 bivalents in diakinesis and metaphase I of meiosis. No sex chromosomes could be identified.

Figs. 1 and 2 are prometaphase and metaphase plates of spermatogonial nuclei of *Microhyla*. By studying a number of spermatogonial metaphase plates 7 pairs of metacentric chromosomes and 6 pairs of acrocentrics have been observed. The

metacentric chromosomes with equal arms have been termed V-shaped while the chromosomes with unequal arms have been called J-shaped. There are 5 pairs of V shaped and 2 pairs of J-shaped chromosomes (Fig. 2).



FIGS. 1-4

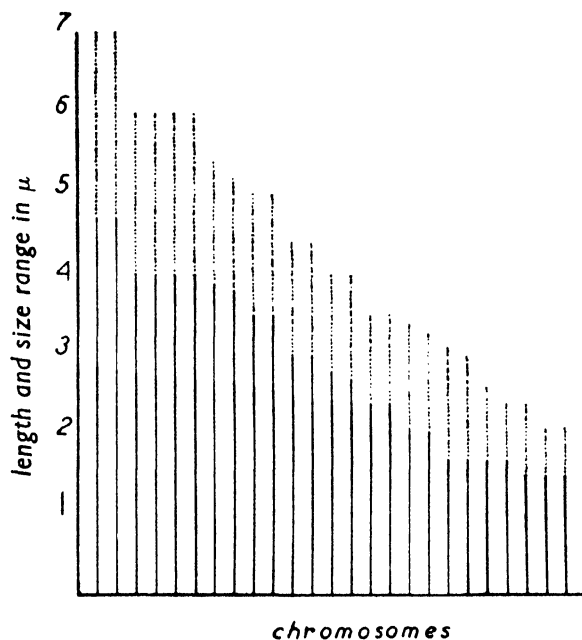
Fig. 1. Prometaphase and Fig. 2 metaphase plates of spermatogonial nuclei of *Microhylla rubra*. $\times 2,850$. Feulgen.
 „ 3. Prometaphase and Fig. 4 metaphase plates of spermatogonial nuclei of *Ramanella variegata*. $\times 2,850$. Feulgen.

It may be mentioned that the four J-shaped chromosomes are longer than the metacentrics with equal arms. The size range of the four chromosomes shown in Fig. 2 is $4.0-4.7\mu$. The lengths of the ten metacentrics with equal arms vary from 2.4 to 4.0μ . The six pairs of acrocentrics range from 1.4 to 3.9μ . Among these the chromosomes of one pair measure 3.8 and 3.9μ respectively. Two pairs are of the same length and measure 1.6μ . The total length of all the twenty-six chromosomes is 72.9μ .

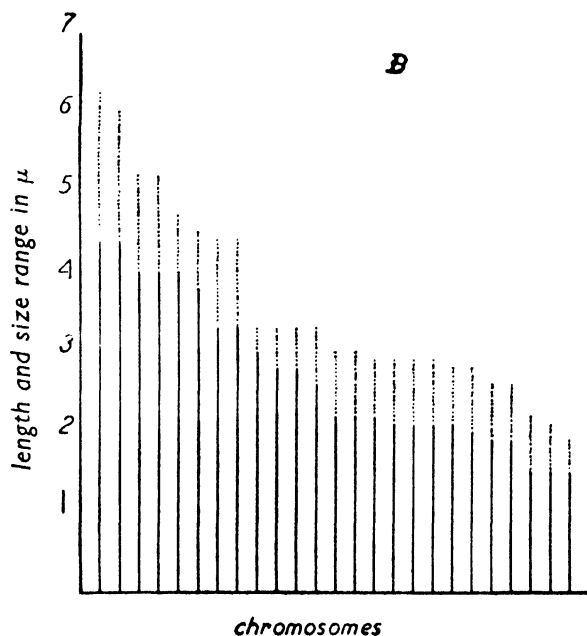
The lengths of the chromosomes in six spermatogonial metaphase plates have been measured and a composite graph A has been drawn indicating their maximum and minimum lengths. In all the six plates, the J-shaped chromosomes are longer; the size range is 4.0 to 7.0μ . The metacentrics with equal arms vary from 2.4 to 6.0μ . Two pairs of acrocentrics are of the same length and the size range for the six pairs is 1.4 to 5.4μ .

Figs. 3 and 4 are prometaphase and metaphase plates of spermatogonial nuclei of *Ramanella variegata*. There are eight pairs of metacentrics and five pairs of acrocentrics.

It may be noticed that the two pairs of J-shaped chromosomes are longer than the V-shaped metacentrics and measure $4.0-4.4\mu$ but the third J-shaped pair is 3.3μ in length. The metacentrics with equal arms vary from 2.4 to 4.0μ . The five pairs of acrocentrics vary from 1.4 to 2.2μ . The total length of the 26 chromosomes in *Ramanella* is 71.5μ . The composite graph B represents the size range of the chromosomes of *Ramanella*, as seen in six spermatogonial plates. The two pairs of J-shaped chromosomes vary from 4.0 to 6.3μ and the third pair, 3.3 to 4.4μ . The V-shaped metacentrics range from 2.1 to 4.7μ while the acrocentrics vary from 1.4 to 2.9μ .



Graph A illustrates the length variations of the 26 spermatogonial chromosomes in *Microhyla rubra* in six metaphase plates examined. Scale 1 cm.—1 μ .



Graph B illustrates the length variations of the 26 spermatogonial chromosomes in *Ramanella variegata* in six metaphase plates examined. Scale 1 cm.—1 μ .

On comparing the lengths of the chromosomes of the two forms, it is found that the smallest acrocentric measures 1.4 μ in both; but the size range of the acrocentrics is 1.4–2.9 μ in *Ramanella* and 1.4–5.4 μ in *Microhyla*. The number also varies; six pairs in *Microhyla* and five pairs in *Ramanella*. One pair of J-shaped

chromosomes is longer than the V-shaped metacentrics in both; 3 pairs of J-shaped chromosomes are found in *Ramanella* while only two pairs are observed in *Microhyla*.

The number of the metacentrics with equal arms is the same in both species.

Table 1 shows the size range and shapes of the 13 pairs of homologous chromosomes of *Microhyla rubra* and Table 2 shows those of *Ramanella variegata*. Table 3 summarizes the important differences between the two species.

TABLE 1

Table showing size range and shapes of the 13 pairs of homologous chromosomes of *Microhyla rubra*

<i>Microhyla rubra</i>		Diploid No. 26
Pair 1	4.7-7.0 μ	J-shaped.
Pair 2	4.0-6.0 μ	J-shaped.
Pair 3	4.0-6.0 μ	V-shaped.
Pair 4	3.5-5.0 μ	V-shaped.
Pair 5	3.0-4.4 μ	V-shaped.
Pair 6	2.7-4.0 μ	V-shaped.
Pair 7	2.4-3.5 μ	V-shaped.
Pairs 8, 9, 10, 11, 12 and 13	1.4-5.4 μ	Acrocentric.

TABLE 2

Table showing size range and shapes of the 13 pairs of homologous chromosomes of *Ramanella variegata*

<i>Ramanella variegata</i>		Diploid No. 26
Pair 1	4.4-6.3 μ	J-shaped.
Pair 2	4.0-5.2 μ	J-shaped.
Pair 3	3.8-4.7 μ	V-shaped.
Pair 4	3.3-4.4 μ	J-shaped.
Pair 5	2.8-3.3 μ	V-shaped.
Pair 6	2.6-3.3 μ	V-shaped.
Pair 7	2.2-3.0 μ	V-shaped.
Pair 8	2.1-2.9 μ	V-shaped.
Pairs 9, 10, 11, 12 and 13	1.4-2.9 μ	Acrocentric.

TABLE 3

Table showing the differences between the chromosomes of *Microhyla rubra* and *Ramanella variegata*

	<i>Ramanella variegata</i>	<i>Microhyla rubra</i>
Chromosome number ..	26	26
Number of large metacentric chromosomes	8	12
Number of small metacentric chromosomes	8	2
Number of acrocentric chro- mosomes	10	12
Size range, metacentrics ..	2.1-6.3 μ	2.4-7.0 μ
Size range, acrocentrics ..	1.4-2.9 μ	1.4-5.4 μ
Total size range	1.4-6.3 μ	1.4-7.0 μ

SUMMARY AND CONCLUSIONS

The diploid number of chromosomes in *Ramanella variegata* and *Microhyla rubra* is 26. There are 8 pairs of metacentric chromosomes and 5 pairs of acrocentrics in *R. variegata* whereas there are 7 pairs of metacentrics and 6 pairs of acrocentrics in *M. rubra*. The chromosome lengths of the two species in mitotic metaphase have been measured.

The main differences between the chromosomes of the two species are:

(a) There is one more metacentric chromosome (J-shaped) in *R. variegata* than in *M. rubra*.

(b) The total length of the 26 chromosomes is 72.9μ in *Microhyla* and 71.5μ in *Ramanella*.

It is difficult, with the available information, to evaluate the significance of these differences or to account for them. Morphologists believe that *Microhyla* is perhaps more primitive than *Ramanella* (Noble, 1931) and chromosome analysis of more species of this family is desirable before any conclusions can be drawn.

ACKNOWLEDGEMENT

I am thankful to Professor B. R. Seshachar for suggesting the problem and for his guidance and encouragement.

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MORPHOLOGY OF *PALLAVICINIA* WITH REFERENCE TO ITS SPECIES PROBLEM AND THE INDIVIDUALITY OF *PALLAVICINIACEAE*¹

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Pallavicinia with about 40 species is spread over tropics of both the hemispheres. Stephani (1900–24) divides it into two sections: Procumbentes (*Eupallavicinia*) and Dendroideae (*Mittenia*). In India it is represented by 7 species with restricted distribution (see Chopra, 1943).

The present investigation gives a morphological account of *P. ambigua* (Mitt.) St., *P. Lyellii* (Hook.) Gray and *P. longispina* St. The development of sex organs has been studied in detail in *P. ambigua* and *P. Lyellii*. In case of *P. longispina* a study of archegonium was made from herbarium specimens and the stages available do not suggest any marked deviation from the general plan for the genus. Since the development of sex organs follows the usual plan described for other Anacrogynous Jungermanniales it is thought desirable to omit the figures so common in literature. A study of variations of certain characters of thallus including the structure of antheridial scales (except in *P. longispina*, where male plants were not available), involucre and perianth has been undertaken in each species to determine their bearing on the species problem of the genus.

P. ambigua, collected from Travancore, India (through courtesy of Mr. S. Nayar, to whom thanks are due), grows along moist cuttings and sheds spores by the end of monsoons. During the unfavourable periods the thalli, as in other species of the genus, lose their wings except towards the growing tips, become thickened, hard and dark brown. These parts form the basal prostrate region while they resume growth and develop aerial assimilatory shoots. *P. Lyellii*, collected from various places from Assam and Japan (through courtesy of Dr. S. Hattori), is restricted to moist protected places adhering to stones or along banks of streams or on moist hill slopes. In Japan it also grows on soil rich in humus and epiphytically on bases of *Cryptomeria japonica* (Hattori, personal communication). *P. longispina* collected from various places in Japan (by Dr. S. Hattori) occurs on humus, exposed and moist rocks, fluffy banks, moist soil and stream banks.

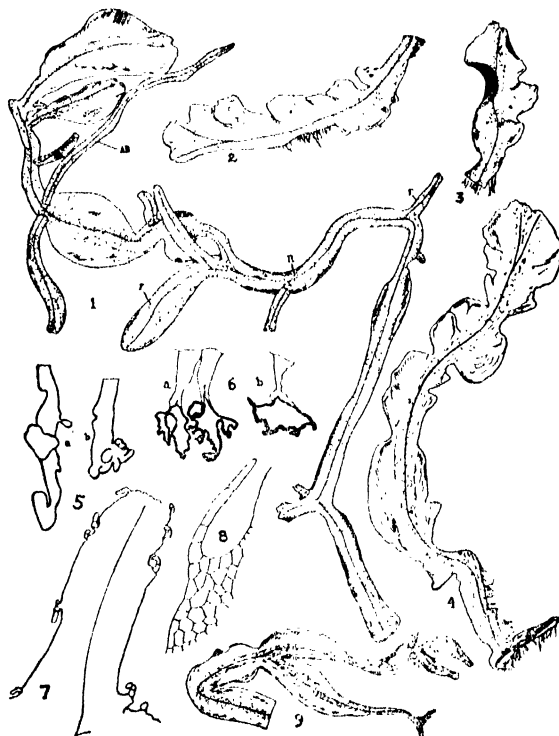
EXTERNAL FEATURES

The species are unisexual, occur in patches, are translucent pale to yellowish-green, simple or branched and prostrate as in *P. levieri* and *P. radiculosa*. *P. longispina* sometimes shows the habit (Fig. 10) exhibited by *P. zollingeri* (Smith, 1938). All species have an ascending, more or less flat, winged region and a basal wingless rhizomatous region (Fig. 1). The former have the margin entire, wavy, undulate or with indistinct or distinct leaf-like lobes (Figs. 2–4); and the latter is

¹ This investigation was carried out at Gauhati University, Assam, India.

formed by the suppression of the wings caused by non-availability of light or, due to their being covered by the ascending winged region, they serve to bring the latter into light. Rhizoids are borne only on the midrib region, unlike that in *P. levieri* (Campbell, 1939) where they arise from the entire ventral surface. They harbour a number of unicellular and filamentous algae. In *P. Lyellii* often they are localized (Fig. 9) and the rest of the midrib lacks them. They are yellowish-light brown, long, thick walled, with sucker-like tips (Fig. 5), and in epiphytic forms shorter, with broad suckers (Fig. 6); and thin, long, without suckers—the former for fixation and the latter for absorption. The midrib shows a conspicuous mid-dorsal vein, becoming black with age, and with a broad one cell thick, longitudinal wing on its either side. The former consists of conducting tissue and the latter is entirely photosynthetic with cells, 4–6 angled, thin or nearly thickened.

The thalli in *P. ambigua* are up to 5 cm. long, 4.5 mm. broad, with the midrib region about 0.5–1.5 mm. broad and nearly convex on the ventral surface, when young bearing 1–3 cell long hairs (Fig. 7) along the thallus margin—more aggregated towards the apical notch, becoming stouter in the older regions and mostly drying up or decaying off due to low moisture; in *P. Lyellii* they are up to 7 cm. long, 5 mm. broad, with the mid-dorsal vein about 0.5 mm. broad and in *P. longispina* they are up to 5 cm. long, about 6 mm. broad, with the midrib region 0.5–1 mm. broad, occasionally indistinctly concave, the margin of the thallus bearing



FIGS. 1–9.

- Fig. 1. *P. ambigua*, thallus with basal rhizomatous region (R), young adventitious branches (AD) and forkings (F). $\times 3\frac{1}{2}$.
 Figs. 2–4. Same showing thalli with lobes and leaf-like margins. $\times 3\frac{1}{2}$.
 Fig. 5. Apices of rhizoids in *P. ambigua* and *P. Lyellii*. $\times 100$.
 „ 6. Rhizoids of epiphytic forms of *P. longispina*. $\times 100$.
 „ 7. Apex of a lobe of *P. ambigua* showing marginal teeth. $\times 35$.
 „ 8. A marginal tooth from thallus of *P. longispina*. $\times 35$.
 „ 9. Thallus of *P. Lyellii* (note forking, walking habit and localization of rhizoids). $\times 3$.

3-6 cell long, persistent hairs or spines (Fig. 8)—more aggregated towards the apex, white to yellowish in colour and becoming light brown with age. The epidermal cells and the cells towards the middle of thallus are variable in size, even within the same species. In all the species studied here the surface cells of the vein are polygonal broad-linear, usually with highly thickened cells and about $45 \times 30 \mu$ or $60 \times 18 \mu$.

Forking of the thallus is caused by a vertical division in the apical cell. Such forkings may have a limited or unlimited growth, depending on the available light and moisture conditions. Often those developing archegonial receptacles may stop further growth. Adventitious branches are formed profusely as ventral innovations from the midrib region and in their formation there is not a forking of the apical cell; they are cylindrical to begin with but sooner or later develop wings, first around the apex. Usually the basal region retains the cylindrical form, distinctly or indistinctly. Each in turn develops one to many ventral innovations and a single plant may become as much as 7 cm. in diameter. It is, however, interesting to note that the first adventitious branch and those developed from it in turn are formed from the same side of the wings as that of the first branch from the thallus; in other words if the first adventitious branch is formed from the left side of the thallus, those developed from it in turn will also be formed from the left side of the respective branches bearing them.

Perennation and vegetative propagation: *P. Lyellii* and *P. longispina* perennate with help of apical tubers. The latter represent the swollen apices and are formed at the end of the growing season. They are cylindrical and profusely covered with thick-walled, pale pinkish-brown rhizoids (Fig. 11); usually underground or in the epiphytic forms burying themselves in the substratum formed by mosses. In *P. longispina* more than one may be formed per thallus and the plant may thus assume a 'walking habit' (Fig. 12; an occasional case figured for *P. Lyellii*, Fig. 9). In all the species perennation is also by the rhizomatous region which is dormant during unfavourable periods.

Vegetative propagation is mostly by fragmentation of adventitious branches, or of the basal rhizomatous region; in *P. longispina* also by formation of 'stolon-like' structures (Fig. 11) which are such adventitious branches that lose their wings, become cylindrical, long (up to 3 cm.), thick and have a somewhat burrowing habit. Later they develop wings along the apex.

INTERNAL STRUCTURE

The midrib of the thallus is usually about 10-11 cells thick in *P. ambigua*, 12-14 cells in *P. Lyellii* and 12-30 cells in *P. longispina*. It suddenly passes into the wings, one cell thick and with thin-walled polygonal cells. The central conducting region consists of long, narrow, thick-walled cells running parallel to the mid-dorsal vein, about 6μ wide and pitted as in other species of the genus (Campbell and Williams, 1914) and *Symphyogyna* (Finlayson, 1950). It serves for conduction of solutes and a branch from it may or may not be given to the female receptacle. It has no connexion with the conducting region of adventitious branches. It is, however, connected with the branches formed as a result of forking. Here it divides far in advance of the forking. The region below it is parenchymatous and in the older thalli infested with a mycorrhizic fungus; and becoming light brown together with the adjacent cells later on. The mycorrhizic fungus is inter- and intra-cellular (Fig. 13b) and enters the thallus through the rhizoids (Fig. 13a). It spreads in the cells just below the conducting strand and rarely in those towards the dorsal surface but never in the receptacles. They are neither symbiotic nor obligatory for the development of the thallus. The young adventitious branches lack them. The wingless rhizomatous region is nearly spherical in a cross-section and consists of parenchymatous polygonal cells, full of starch and fungal hyphae and with the

outer cells thick walled. In stolons such cells develop chloroplasts, are polygonal, thin walled and lack fungal hyphae when young.

Apical cell: The apical cell is lodged within the apical notch and is protected by a number of 3-5 celled hairs with mucilaginous tips (Fig. 14). It is two sided as in most of the other species of the genus (Campbell and Williams, 1914; Haupt, 1918), *Riccardia pinguis* (Clapp, 1912), *R. indica* (Kachroo, 1953), *Metzgeria himalayensis* (unpublished data) and *Hymenophyllum* (Goebel, 1905). It cuts off segments alternately right and left, each of which is cut into an inner cell and an outer cell—the former contributes to the thick midrib and the latter to the cells of the wing. Farmer (1894) reports an apical cell with 3 cutting faces in *P. decipiens*.

MALE PLANT

They are much narrower than the female plants in contrast to *P. radiculosa* where both are equal in size (Smith, 1938); usually about half in breadth and nearly equal in length; narrowly linear and short-lived. They may fork once or twice at the apex (Fig. 16). In *P. ambigua* often the midrib region is prolonged into a thin flagellum-like shoot with a bunch of rhizoids at the apex, probably serving for vegetative propagation (Fig. 15). Antheridia are borne on each side of the midrib either in continuous series or in series interrupted here and there by sterile areas. Each (Fig. 16) or a group of 2-3 is subtended by a scale arising from the midrib. The latter is nearly triangular in outline (Fig. 15b); having shortly laciniate margin in *P. ambigua*; nearly roundish-ovate and shortly laciniate in *P. Lyellii* and more or less similar in *P. longispina*.

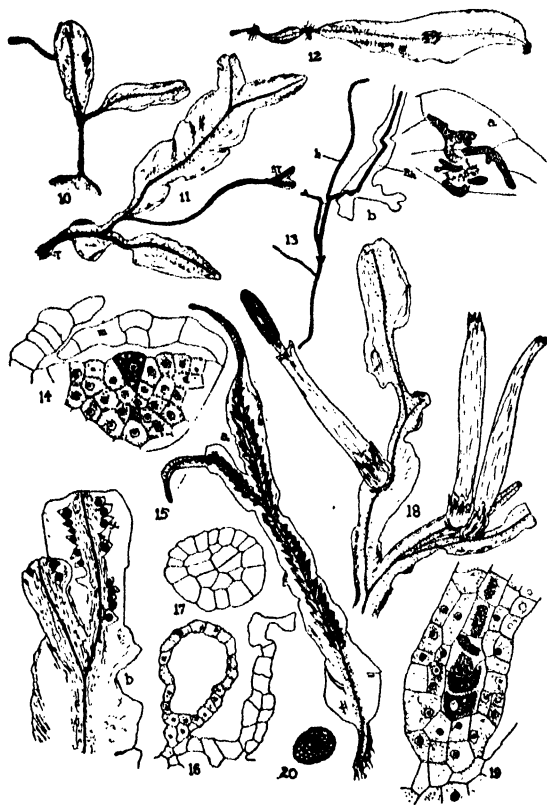
The antheridium is shortly stalked, nearly globular, with a single layered wall (Fig. 16), green when young and becoming orange at maturity. It resembles in its development the other Anacrogynous Jungermanniales. The initial makes its appearance as a papilla which soon enlarges and undergoes a transverse division to form an upper primary antheridial cell and a larger stalk cell. The latter enlarges and undergoes a transverse division to form a stalk, 2 cells long. Subsequently one or more transverse walls appear forming a stalk 3-4 cells long. Occasionally the original basal cell may undergo a vertical division before any transverse walls appear. It, however, remains short and is never more than 4 cells thick. The primary antheridial cell divides by a nearly vertical wall into 2 nearly equal halves. In each of the 2 primary antheridial cells 2 periclinal walls at right angles to each other are laid down, resulting in the formation of 4 peripheral cells and 2 primary spermatogenous cells. The latter divides by a transverse wall followed by a vertical one, appearing first in the lower half, or 2 vertical walls laid one above the other; dividing each spermatogenous cell into 4 quadrants (Fig. 17). The latter after regular vertical and transverse divisions form numerous spermatocytes, 4-5 sided in outline, densely laden with cytoplasm and each dividing diagonally to form 2 biciliated spermatozooids. The antheridial wall is one layered, chlorophyllous when young, becoming orange at maturity and breaking irregularly at the apex during dehiscence. The spermatozooids are liberated in a mucilaginous mass, are coiled, with 2 long cilia at the anterior end and a small vesicle at the posterior end.

FEMALE PLANT

They are as large or larger than the vegetative plants. Archegonia develop in groups in restricted areas, female inflorescences (Frye and Clark, 1937) or receptacles (Smith, 1938) at various points on the dorsal surface of the thallus along the midrib (Fig. 18). They are formed near the apex of a shoot but do not check its growth. Each is about the midrib in diameter and there is no correlation between their position and dichotomy of the thallus as in *P. zollingeri* (Campbell, 1939) but they might occur at each branch of the dichotomy; usually solitary at a point but

in *P. Lyellii* occasionally met with in a series. Around the receptacle grows a sheath, the involucre, from the surface of the thallus. It is short, cupulate, irregularly lobed; mediumly 1-2 lacinate in *P. ambigua*, unequally lacinate with each lacina comparatively thinner, forked and more branched in *P. Lyellii* and broadly lacinate in *P. longispina*.

During the development of archegonium a papilla-like cell makes its appearance from the receptacle surface and soon undergoes a transverse division usually to form a larger, rarely smaller or nearly equal, basal cell and an upper archegonial initial. The latter is cut off by 3 intersecting vertical walls into an axial cell and 3 peripheral cells. The axial cell divides by a transverse wall into an upper cover cell and a central cell. The latter undergoes another transverse division cutting off nearly equal cells, the upper neck canal cell and the lower ventral cell; but the divisions in the two cells are not simultaneous. Later divisions are similar to those



FIGS. 10-20.

- Fig. 10. *P. longispina* showing erect habit. $\times 2\frac{1}{2}$.
 11. Same showing a stolon (ST) and a tuber of the previous season (T). $\times 2\frac{1}{2}$.
 12. Same with walking habit. $\times 5$.
 13a. Mycorrhiza (inter- and intra-cellular) in ventral tissue of thallus. $\times 212\frac{1}{2}$.
 13b. Same, branched and entering a rhizoid (h hypha, Rh rhizoid). $\times 100$.
 14. Apical cell in L.S. (m mucilage hair). $\times 162\frac{1}{2}$.
 15a. *P. ambigua* (male plant). $\times 2$.
 15b. Same, apical region only shown (a, antheridium; sc, scale). $\times 5$.
 16. *P. ambigua*, mature antheridium and a scale in L.S. $\times 16$.
 17. Same, T.S. young antheridium. $\times 162\frac{1}{2}$.
 18. Female plant of *P. ambigua*. $\times 2\frac{1}{2}$.
 19. V.S. archegonium (basal portion only shown), *P. ambigua* showing two ventral canal cells and two eggs within the venter. $\times 162\frac{1}{2}$.
 20. Spore of *P. Lyellii*. $\times 162\frac{1}{2}$.

described for other Jungermanniales (Campbell, 1939; Finlayson, 1950; Mehra and Vasisht, 1950; Mehra and Khanna, 1950). The neck is elongated and more or less twisted in the mature archegonium. The number of neck cells varies from 5-11, with 8-23 cells in each row of the neck. The neck canal cells in the mature archegonium are 9-13 in contrast to 5-6 in *P. radiculosa* (Campbell, 1939). The ventral canal cell is angular, spherical or disc-shaped, and usually smaller than the egg or rarely more or less equal in size. Rarely 2 ventral canal cells and eggs are observed within a venter in *P. ambigua* (Fig. 19). Two eggs also occur in *Petalophyllum indicum* (Mehra and Vasisht, 1950) where one of the ventral canal cells behaves as the second egg. The venter becomes 2-3 layered after fertilization. The basal cell of the archegonium forms 2-6 cell long stalk after undergoing transverse and vertical divisions.

The involucre develops from the surface of the thallus around the group of archegonia in the form of a ring of scales which become united due to basal zonal growth and remain free at the apex, where each is lacinate. When mature it is 2-3 cells thick at the base and one cell at the apex.

The development of the perianth is stimulated if a single or more archegonia are fertilized. It grows next to involucre, is 3-4 cells thick at the base, cells in mature condition are thick walled, 4-6 sided, elongated and long. Sometimes it forms inside 1-4 knobs projecting inwards and up to 3 cells broad. At maturity it is tubular and covers the young sporogonium completely.

Just before fertilization the neck canal cells and the ventral canal cell become mucilaginous and presence of moisture brings about their distension which forces apart the 4 lid cells, each of which usually bends outwards to create a cup-like depression or occasionally the force of distension is so great that it forces the lid cells to break off from the neck. The spermatozoids enter in a mass but ultimately one fertilizes the egg.

SPOROAGONIUM

A single sporogonium develops per receptacle due to nutritional competition, though more than one or all archegonia may be fertilized. The fertilized egg becomes nearly spherical in outline, enlarges, delimits its own wall and completely fills the venter. It forms a 3 celled filamentous embryo which is later differentiated into a foot, seta and a capsule. The foot is distinctly demarcated from the seta unlike in *P. radiculosa* (Campbell, 1939) where it merges imperceptibly into the base of the seta. It is about 1 mm. long in the young sporogonium, composed of parenchymatous cells with dense protoplasmic contents in the peripheral cells which are haustorial in nature. The seta is circular in outline, in a cross-section composed of 9 cells, made up of numerous elongated cell rows; cells thin walled, usually chlorophyllous and in the younger stages filled with starch grains. In a L.S. the cells are much compressed, broader than long but in the mature condition they elongate and attain a length several times their original. The seta is slender, whitish, 5-7 cm. long in *P. ambigua*, 3-4-5 cm. in *P. Lyellii* and *P. longispina*. Rarely its peripheral region develops a few false air spaces by disintegration of the adjoining cell walls; they bulge outwards and open to the exterior by their entire surface. The seta provides a good substratum for the growing sporelings dehiscence from the capsule and for filamentous algae.

The capsule is oblong cylindrical, green when young, reddish-brown at maturity, 5 mm. long, 0.5-1 mm. in diameter, with 2-3 layered wall—the inner getting resolved and the outer becoming dark brown with development of thickenings towards their inner angles. The elaters are attenuated, thin walled, with 2-3 spirals; up to $600 \times 6\mu$ in *P. ambigua* and $200 \times 5\mu$ in the other two species. Some of them remain attached to the base of the capsule. Spores are spherical, light-reddish brown, with an indistinct triradiate mark and finely reticulate exine

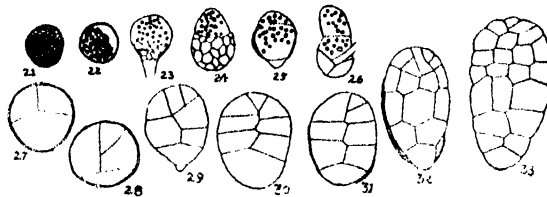
(Fig. 20); $12-16\mu$ in *P. ambigua*, $12-28\mu$ in *L. Lyellii* (with margin papillate) and about 12μ in *P. longispina*.

The calyptra ensheathing the young capsule is thin, slender, 2 cells thick, with the cells much longer than broad.

When mature the capsule is gradually pushed up due to the elongation of the seta and pierces through the calyptra finally coming above the surface of the thallus. It dehisces along (2), 3, (4) valves at maturity, the latter remaining coherent at the apex. Their splitting starts at the base of the capsule and just reaches the apex where all the cells are highly thickened. This prevents their complete separation.

SPORE GERMINATION

The spores of *P. ambigua* and *P. Lyellii* germinate while still within the capsule as in *Pellia*, *Porella* and *Conocephalum* (Chaloud, 1932) and *Dumortiera* (unpublished data). Wolcott (1942) does not describe intracapsular germination in *P. Lyellii* collected from Texas. (Germination is not studied in *P. longispina*). Each spore enlarges slightly or greatly as evidenced by distension of reticulations (Fig. 21); the chloroplasts aggregating to one side (Fig. 22)—probably towards the side facing capsule wall; leaving a small clear space towards the opposite end as in *Pellia* (Goebel, 1905). The latter is cut off by a wall and forms the first rhizoid (Fig. 23). The enlargement of spore results in an irregular rupture of exine, either to liberate the germ papilla (Fig. 24) or the rhizoid (Fig. 25) or the cell mass develops inside (Fig. 30). Occasionally the exine ruptures along the indistinct triradiate mark and the germ papilla elongates into a germ tube (Fig. 26). The upper half of the germ cell now undergoes a vertical division (Fig. 27) and later a wall is formed inclined obliquely to the long axis of the germ cell to form an apical cell with two cutting faces (Fig. 28) as in *Metzgeria* (Goebel, 1905) and *Riccardia indica* (Kachroo, 1953), or the upper cell may undergo a few transverse divisions before a vertical wall appears and an apical cell is formed due to formation of two walls oblique to each other. Activity of this cell results in an enlarged cell mass (Figs. 29-33) and it is gradually lodged within an apical notch, where mucilage papillae are formed from the surrounding marginal cells of the thallus to protect the growing region.



FIGS. 21-33. Stages in germination of spores up to formation of cell mass in *P. ambigua* and *P. Lyellii*. Figs. 21-25, 27 and 28 intracapsular and rest from soil culture. $\times 209$.

Variations in certain characters of the thallus, organs associated with sex organs and their bearing on species problem in the genus :

Thallus : The great deal of variation in the species embraces their vegetative growth, both while growing under different habitats (Table 1 and Fig. 34), altitudes (Tables 2 and 3) and within the same community (Figs. 35-37). It appears that substrate exerts considerable influence on the growth (Fig. 34). Thus large numbers of conducting cells occur in the epiphytic forms due to conservation of water and this might also speak for less breadth of the thallus to check excessive transpiration; in those living on humus the need for conservation is far less, consequently this region occupies a comparatively smaller area of the midrib and the largest breadth of the thallus (in this case) might be due to prolific vegetative growth under congenial conditions of life. The next congenial habitat is shady soil.

TABLE 1

*Variation in vegetative growth of P. Lyellii under different habitats**

Habitat	Thallus breadth	Thallus thickness		Epidermal cells		Area C.S. in T.S.	Number of cells in T.S.	
		Midrib	Wing	Marginal	Middle		Midrib	C.S.
	mm.	μ	μ	μ	μ	μ		
A. On rotten wood	3-3.2	214.4	48	32 \times 89 μ	64 \times 64 68 \times 60	64 \times 54	33	12
B. Epiphytic	2.5-5.1	304	48	28.8 \times 57.6	46 \times 46 28.8 \times 41.6	64 \times 76	45-90	12
C. On soil rich in humus	6	320	54.4	22.4 \times 70.4 57.5 \times 89	57.6 \times 89	48 \times 60.8	36	12
D. Shady banks	4	384	48	22.4 \times 73.6	44.8 \times 64	64 \times 105	60	15
E. Moist hill slopes	4	304	46	27.2 \times 57.6	41.6 \times 32	51 \times 64	24	13

* Plants with 1 or more female receptacles are regarded here as well developed thalli. Each reading given is an average for 5 thalli.

The readings of thickness of thalli, epidermal cells and conducting region have been taken at corresponding places in the thalli of various groups.

C.S.—Conducting strand, midrib does not include conducting strand (same details for other tables). T.S.—Transverse section.

The growth of *P. Lyellii* and *P. longispina* under different altitudes (Tables 2 and 3) shows that the species are not fundamentally affected as far as their vegetative features are concerned and the latter, therefore, do not show any correlation with the change of altitude. The variations exhibited are those which are expected even while they grow in a single community. However, this requires further study and a care in collection of the various forms at different altitudes.

TABLE 2

Variation in vegetative growth of P. Lyellii under different altitudes in Japan (Province, Hyuga—collection, Hattori, Jan.—Nov. 1950)

Altitude	Thallus breadth	Thallus thickness		Epidermal cells		Area C.S. in T.S.	Number of cells	
		Midrib	Wing	Marginal	Middle		Midrib	C.S.
m.	mm.	μ	μ	μ	μ	μ		
15	5	99	11.25 39 \times 33	108 \times 24	105 \times 45	15 \times 15	14	46
30	3	75	11.4	39 \times 33 93 \times 27 57 \times 30	75 \times 39 90 \times 48 63 \times 54	30 \times 18	15	55
150	6.1	51	11.4	84 \times 24 45 \times 21	57 \times 24 30 \times 51	15 \times 18	14	28
250	5.2	63	12	75 \times 18 42 \times 21	75 \times 33 54 \times 33	15 \times 20	11	22

TABLE 3

Variation in vegetative growth of P. longispina growing on moist rocks under various altitudes in Japan (collection, Hattori, 1950)

Altitude	Thallus breadth	Thallus thickness		Epidermal cells		Area C.S. in T.S.	Number of cells	
		Midrib	Wing	Marginal	Middle		Midrib	C.S.
m.	mm.	μ	μ	μ	μ	μ		
5	2	176	36.8	54.4 × 19.2	28.8 × 35.2	48 × 32	12	36
20	2.5-3.5	320 (480)	38.4	80 × 28.8	35.2 × 48	76 × 106	20	74
400	4	288	38.4	70.4 × 22.4	64 × 44.8	38 × 80	15	63
700	4-6	256	38.4	48 × 22.4	38.4 × 86.4	48 × 73	15	56
800	4-4.5	256	40	80 × 19.8	74.6 × 38.4	48 × 35.2	16	36

The study of the species within a community (Figs. 35-37) illustrates that no two features usually show a correlation between them and that variability appears to be an inherent feature for each individual of the various species. (It is probable that microclimate has a dominating hand in shaping the individual). The epidermal cells, whether marginal or those towards the middle, are highly variable (Table 4) in size; so are the cells in the midrib and the area of the conducting strand.

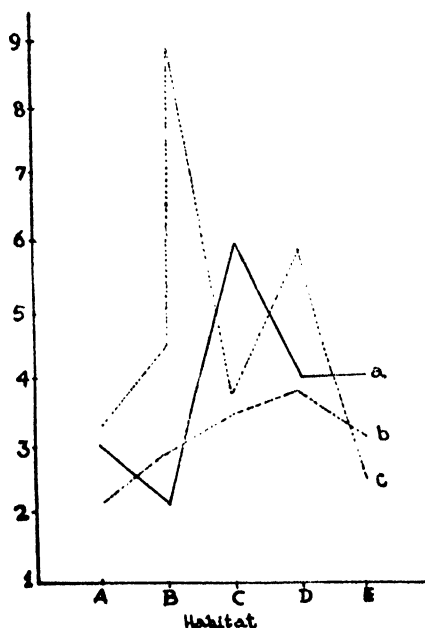
TABLE 4

Variability in epidermal cells of thallus

Plants	<i>P. ambigua</i>		<i>P. Lyellii</i>		<i>P. longispina</i>	
	Marginal	Middle	Marginal	Middle	Marginal	Middle
	μ	μ	μ	μ	μ	μ
1	48.4 × 28.5	54.1 × 28.5	51.3 × 31.3	100.9 × 39.9	39 × 30	57 × 39
	65.5 × 25.6	71.2 × 25.6	72.2 × 19.9	57 × 42.7	66 × 27	96 × 30
2	45.6 × 18.5	51.3 × 34.2	39.9 × 18.5	76.4 × 25.6	54 × 24	48 × 39
	57 × 25.6	76.4 × 25.6	27 × 25.6	28.5 × 51.3	60 × 21	66 × 33
3	51.3 × 31.3	57 × 18.5	42.7 × 31.3	51.3 × 39.9	45 × 24	57 × 42
	42.7 × 22.8	65.5 × 31.3	79.8 × 28.5	85.5 × 28.5	63 × 24	75 × 33
4	42.7 × 28.5	57 × 25.6	22.8 × 51.8	71.2 × 51.3	30 × 33	57 × 33
	65.5 × 17.1	74.1 × 28.5	28.5 × 57	65.5 × 34.2	63 × 18	75 × 30
5	57 × 25.6	79.8 × 22.8	42.7 × 37	48.4 × 48.4	42 × 33	45 × 24
	39.9 × 28.5	59.8 × 34.2	85.5 × 34.2	71.2 × 28.5	60 × 27	60 × 33

Antheridial scale: Usually a single scale ensheaths a single antheridium and such scales may have either one or more papillate protrusions towards the apex, or these may be prolonged into lacinae (teeth), or have neither; or the scale may be bifid or asymmetrical in outline (see Figs. 38 and 39 for the most common forms of the scales). Scales showing more than one lacina usually ensheath more than one antheridium within and in the case of scales holding their individuality (i.e. not fusing with adjoining ones) there appears to be a correlation between the number of lacinae and the number of antheridia ensheathed within—usually one antheridium per lacina, in *sensu lato*. In case of scales forming flaps this arrangement becomes obscure and it is noteworthy that in such cases usually antheridia do not

occur within the regions where adjoining scales fuse. It is not, however, uncommon to find antheridia unprotected (Fig. 38c), single laciniate scales with more than one antheridium, or many papillate-laciniate scales with a single antheridium or sterile scales. The formation of flaps is due to fusion of the bases of adjoining scales—the apical region being free and marking the number of the constituent scales distinct. Rarely scales are bivalved and appear as cups (Fig. 38o) with one or more antheridium within.



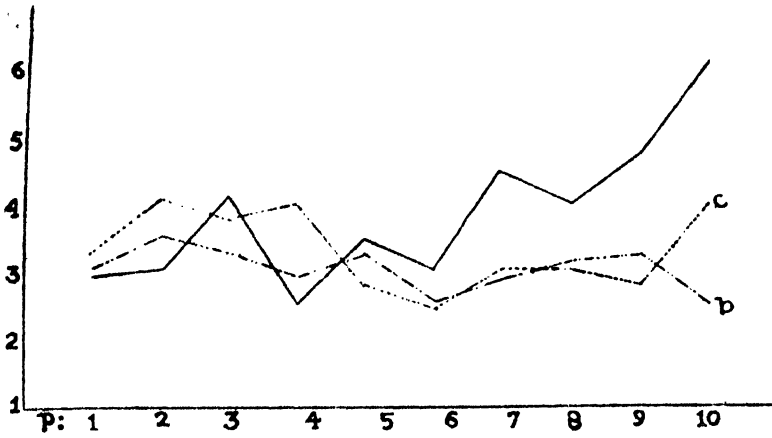
34

FIG. 34. Growth of *P. Lyellii* under different altitudes (a, thallus breadth; b, thickness of midrib; c, number of cells in midrib. A-E as in Table 1).

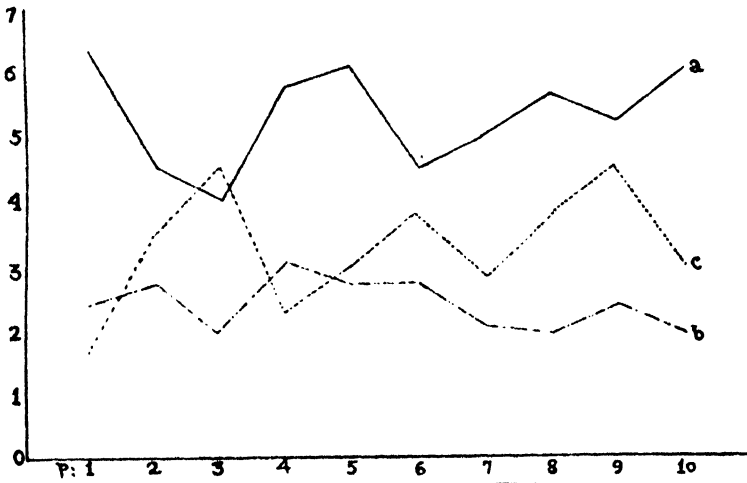
Involucre: Each species shows a great deal of variation in structure of involucre so far as the lacinae are concerned. Thus in *P. Lyellii* the primary lacinae are either thick, short, unbranched or branched, 1-2 times at the base; in form of plates or they are thin, longer, less branched or more branched; the tips becoming glandular (Fig. 40, a-c). Similar variations occur in the other species but there the lacinae are less branched and more or less plate-like, being thus clearly distinguishable from the above type (Figs. 41 and 42).

Perianth: The mouth of the perianth is variable both in opening and in the incidence of lacinae. The mouth may be cut into a few fid or partite lobes, each shortly or broadly laciniate; or the partitions may be deeper or it may be cut into 4 lobes forming a beak-like structure. A few variants are figured for *P. Lyellii* (Fig. 43). Whereas usually the developing sporogonium emerges through the apex of the perianth, lateral emergence is not uncommon (Fig. 43, h).

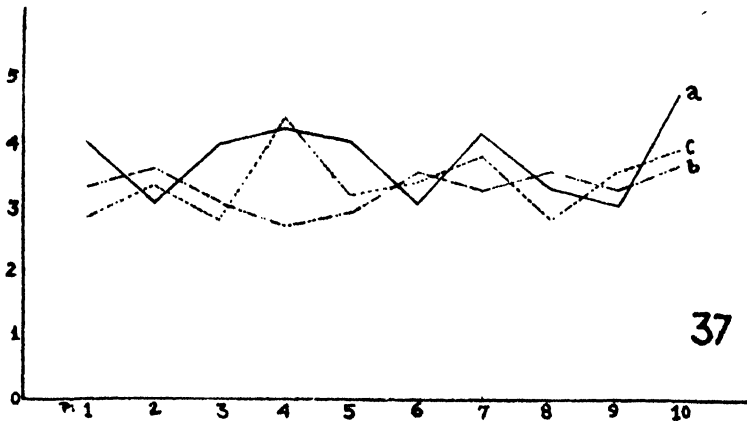
These variations bring forth interesting results involving specific characters and note should be taken of the same while dealing with imperfectly known species (cf. *P. canarus* (vide Pande and Srivastava, 1954) might on this basis be simply a biologic form of *P. Lyellii*) or while describing new species. Cytological investigations might throw more light on this problem.



35



36



37

FIGS. 35-37. Variation in growth of *P. ambigua*, *P. Lyellii* and *P. longispina* respectively growing on moist soil (legend as in Fig. 34); P, plants.



FIG. 38a-q. Variations in antheridial scale in *P. ambigua*. $\times 22\frac{1}{2}$.

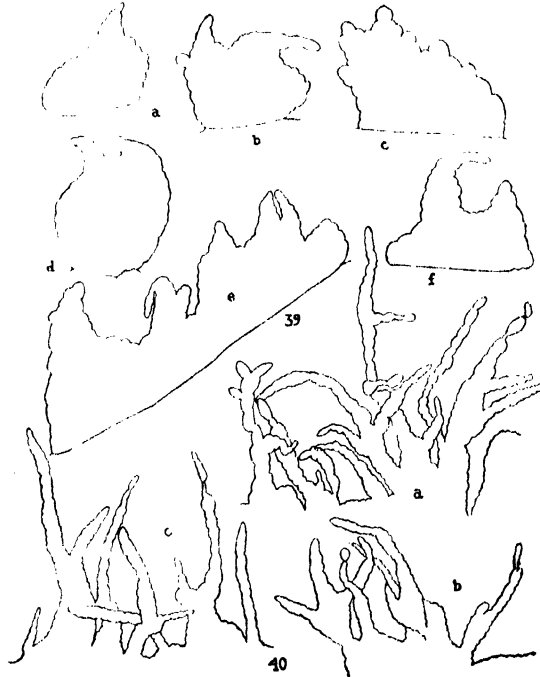


FIG. 39a-f. Variations in antheridial scale in *P. Lyellii*. $\times 22\frac{1}{2}$.

„ 40a-c. Showing variations of lacinae in different involucres of *P. Lyellii* (only a portion shown). $\times 22\frac{1}{2}$.



FIGS. 41-42. Fig. 41a-d. Showing variations of laciniae in different involucre of *P. ambigua* (a portion shown). $\times 22\frac{1}{2}$.
Fig. 42a-b. Same on *P. longispina*. $\times 22\frac{1}{2}$.

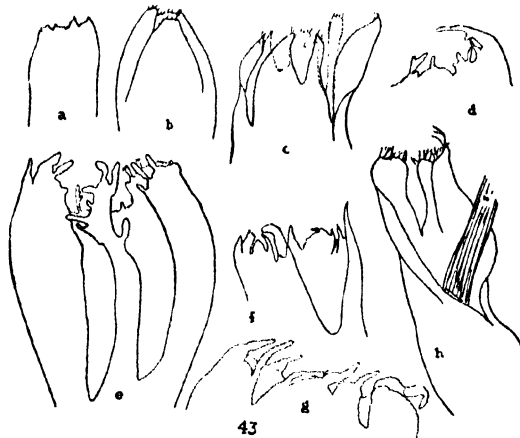


FIG. 43a-g. Showing variations in mouth of perianth and nature of laciniae in *P. Lyellii*; h lateral emergence of sporogonium. a-f $\times 10$; h $\times 22\frac{1}{2}$.

Thus the epidermal cells are not reliable as specific characters, nor are the antheridial scales. But the nature of the involucre, particularly its laciniae (though variable), might be considered reliable for the species to be classified into two groups: those with profuse branched, thin, elongated laciniae (*P. Lyellii* type) and those with less profuse branches and thick plate-like laciniae (*P. ambigua-longispina* type); this goes, to some extent, hand in hand with the presence of the marginal teeth on the thallus, at least in the present case. The mature capsule is too uniform and the

perianth quite variable; the spores also do not show marked inter-specific variation. It is probable that a comparative study of all the 40 species of the genus, on the lines suggested here, might reveal large variation among the species and a number of the latter might turn up to be simply the various biologic forms of but a few species.

Individuality of the family Pallaviciniaceae :

Cavers (1911) in his arrangement of genera under Blyttiaceae places *Pallavicinia* before (i.e. primitive than) *Symphyogyna* and family as number 3 in his division of Anacrogynae. Goebel (1930), followed by Campbell (1939), regards the group as sub-family: Pallaviciniaceae, and places it as ii order in his division of Metzgeriaceae. He placed *Pallavicinia* and *Symphyogyna* only in his sub-family. But like Cavers he recognizes the difficulty of clearly defining these families (Campbell, 1939, p. 121). Verdoorn (1932) includes the genus in his Dilaenaceae which also embraces Goebel's Pallaviciniaceae and Morekiaceae. Frye and Clark (1937) recognize *Pallavicinia* as most primitive member in their Metzgeriaceae and derive it from *Geothallus* through some intermediate form (*F* in their phylogenetic table on p. 104). Smith (1938) regards it as a form between two extreme types: *Riccardia* and *Fossombronia* respectively and remarks 'genera placed in Riccardiaceae have no one feature common distinguishing them from other Anacrogynae (p. 49)'.

In species of *Pallavicinia* studied here, it is usual to have a basal rhizomatous and an ascending photosynthetic region, but it is noted that thalli grown separately under optimum conditions do not form rhizomatous region. Suppression of wings is common and is either on the side facing substratum or on both sides as in *Umbraculum* (Goebel, 1905). The nature and formation of lobes in the thalli is similar to that exhibited by *Symphyogyna*, *Fossombronia* and a few other genera of Anacrogynae. Such developments may have taken place independently in the various families and does not necessarily prelude any near relationships. There is not enough evidence to show that such lobes are leaves, though their presence suggests leafy ancestry.

An inner central conducting strand is also present in *Symphyogyna* (Finlayson, 1950), *Umbraculum*, *Podomitrium* and some species of *Riccardia* and *Metzgeria* (Goebel, 1905). In having an apical cell with two cutting faces the genus resembles *Symphyogyna*, *Riccardia*, *Metzgeria*, *Umbraculum*, *Fossombronia himalayensis* (Pande et al., 1953) and *Calycularia crispula* (Pande and Udar, 1953) but differs from *Petalophyllum indicum* (Mehra and Vasisht, 1950) and *Sewardiella tuberifera* (Mehra and Khanna, 1950) which have tetrahedral apical cell. The intra- and inter-cellular mycorrhiza is a feature common to all the thallose Jungermanniales. Tubers also occur in the related family Codoniaceae, probably due to similar biological adaptations.

The presence of a cup-like involucre is an advance over the single one of *Symphyogyna*, which Goebel (1930) regards as the original one. In *Umbraculum* and *Metzgeria* an involucre is present or absent. In Codoniaceae the involucral bracts are scattered irregularly among the archegonia and are attached at the base. A tubular perianth is also present in *Podomitrium*, *Calycularia* and *Petalophyllum* (Campbell, 1939; Goebel, 1905) but it is absent in *Symphyogyna* and *Metzgeria* (Goebel, 1930). *Riccardia* is peculiar in having neither, and in developing a massive marsupium quite different in origin from calyptra of *Pallavicinia*, this together with the development of an elaterophore is a specialized feature.

The structure and development of sex organs and sporogonium resemble more closely that in *Symphyogyna* (Campbell, 1939; Finlayson, 1950) than with any other genus. The capsule wall is two layered as in *Fossombronia* and unlike that in *Riccardia* and *Calycularia* where it is three layered. A few elaters might remain attached to the base of the capsule. The embryo is filamentous as in *Symphyogyna*.

and *Riccardia*. The dehiscence of capsule is along 2-4 valves which remain attached at the apex as in *Symphyogyna* and in contrast to *Riccardia* where they separate and carry a portion of elaterophore at the apex. The germination of spores shows great similarity with that of *Symphyogyna* and *Pellia*.

Pallavicinia, thus, shares many features with primitive and advanced genera of Anacrogynae, yet retaining its individuality and distinctness from the genera with which it is kept by Verdoorn (1932), Frye and Clark (1937) and Evans (1939). Goebel (1930) and Campbell (1939) are justified in placing it with *Symphyogyna* in a separate family Pallaviciniaceae. With most of the genera it follows parallel lines of development in habit and divergent features in life-history; thus taking view of the contemporary morphology of Hepaticae the various genera or groups of genera should be regarded as end products of long and diversified evolution. This necessitates and justifies the division of Anacrogynae into various families. Each group has further developed special individual features. The intimate relationship between them, however, speaks for a common group of ancestors.

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ABSTRACT

Pallavicinia ambigua, *P. Lyellii* and *P. longispina* occur in patches, usually in moist places. Each has a basal rhizomatous region and an ascending assimilatory region. A mid-dorsal vein and an inner central conducting strand is present. It is connected with that of forking but not with that of adventitious branch. An apical cell with 2 cutting faces is present in the sporolings and the thallus, it cuts segments alternately right and left. Development of sex organs resembles other Anacrogynae. Two eggs and 2 ventral canal cells are observed in an archegonium. The embryo is filamentous. Rarely air spaces develop on the seta. Capsule is oblong-cylindrical and dehisces along 2-4 valves, attached at the apex. Spores germinate while still within the capsule, form a cell mass with 2 sided apical cell. A detailed study of the variations in certain characters of the thallus, organs associated with sex organs, is given and the specific value of the various organs discussed. It appears the species problem leans heavily on the nature of the laciniae on involucre and marginal teeth of the thallus. The retention of the family Pallaviciniaceae with *Pallavicinia* and *Symphyogyna* is suggested.

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THE HEART AND ITS CONDUCTING SYSTEM IN THE COMMON INDIAN FOWL

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INTRODUCTION

An intensive study of the general structure and histology of the avian heart has been generally neglected and there has been much diversity of opinion with regard to the presence of a specialized impulse conducting tissue in it. Keith and Mackenzie (1910) and Mackenzie and Robertson (1910) denied the existence of sinuatrial and atrioventricular nodes in birds. Mangold and Kato (1914) observed a sinuatrial node and an atrioventricular bundle in the heart of the fowl. De Mayer (1952) observed that sinus portions of the conducting tissue disappear in the heart of the adult fowl and that an atrioventricular bundle is either reduced or absent in the sparrow.

A great deal of controversy also exists on the phylogeny of the specialized conducting system in birds and mammals. Davies (1930) pointed out that the atrioventricular connexions present an arrangement which is intermediate between those described by other workers in the fishes and the reptiles and by those in the mammals. Davies and Francis (1941), however, stated that the conducting system of the heart of birds and mammals is a neomorphic development and is not a remnant of the more extensive tissues of similar structure in lower vertebrates. Prakash (1953*a, b*; 1954*a, b, c, d*) upholds the view that the specialized conducting system of the heart of birds and mammals is not a neomorphic development but is a further specialization of similar structures which are present in the heart of lower vertebrates.

The contradictory statements of previous workers on the presence and the phylogeny of the impulse conducting tissue make a further study of the avian heart desirable. In the present investigation the heart of the common Indian fowl has been studied with special reference to its conducting system.

MATERIAL AND METHODS

Six adult specimens of the common Indian fowl, chosen for the present study, were well fed and healthy so as to ensure that there was no deficiency of glycogen content (Davies, 1930) in the various portions of the heart. The hearts were removed from the chloroformed birds and then they were fixed in Bouin's picroformol and embedded in paraffin. Serial transverse and sagittal sections were cut ten micra thick and stained with acid fuchsin.

OBSERVATIONS

Five distinct chambers were found in the heart, viz. a sinus venosus, two atria and two ventricles.

* Contribution No. 1 from Vigyan Mandir, Bhopal.

Sinus venosus: The sinus venosus is a triangular, asymmetrically disposed chamber on the dorsal side of the atria. It is very much reduced because of the invagination in its lateral wall on the right side. Due to this invagination, the cavity of the sinus venosus is divided into a large cephalic and a much smaller caudal portion. The two portions thus formed communicate with each other through a narrow elongated canal. This canal may be referred to as the sinuatrial canal. The right and left precaval veins open into the cephalic portion of the sinus venosus (Fig. 2) while the postcaval vein opens into its caudal portion (Fig. 3). The former discharge almost at the same level, while the latter opens at the level of the sinuatrial orifice.

A short muscular ridge is present (Fig. 3) just above the sinuatrial orifice on the right side of the sinuatrial canal. The ridge is broader at its base and its free tapering end projects in between the two sinuatrial valves. The ridge affects the internal disposition of the sinus venosus in such a way that the postcaval vein appears to open directly into the right atrium. Similarly, the blood which is coming into the cephalic portion of the sinus venosus, through the precaval veins, will be immediately directed by the ridge and the left sinuatrial valve to flow from the sinuatrial canal into the right atrium. The precavals, therefore, appear to pour their blood immediately and directly into the right atrium. Such an arrangement minimizes the importance of sinus venosus and may be responsible for its gradual disappearance in birds and mammals.

The openings of the precaval and the postcaval veins are not guarded by any valves, but they are provided with thick cushion-like outgrowths, developed on the inner surface of the wall of sinus venosus.

The sinuatrial orifice is guarded by two sinuatrial valves which resemble those of reptiles in general structure, although they are situated more dorsolaterally than in reptiles. The right sinuatrial valve is connected at its caudal end with the wall of the right atrium as well as with that of the sinus venosus. The left sinuatrial valve is continuous with the sinus venosus on one side and with the interatrial septum on the other (Fig. 3). Such a connexion, between the sinus venosus and the right atrium through the right sinuatrial valve and between the sinus venosus and the left atrium through the left sinuatrial valve and the interatrial septum, indicates that there exists a continuous muscular path for the conduction of the cardiac impulse, from the sinus venosus to the two atria.

Sinuatrial node: The presence of a sinuatrial node has been observed in many birds and mammals. It is regarded as the site of the initiation of the cardiac impulse. In the fowl this node is present on the cephalic side of the sinus venosus (Fig. 2) between the openings of the right and left precaval veins. The node lies parallel to the wall of the sinus venosus, but it turns cephaloid on the left-hand side. The node is enclosed in a fibrous sheath, is compact and well defined. A number of cells with prominent nuclei are present inside it (Fig. 1). These cells of the node are connected with one another through fine muscle fibres. The S-A node is connected with the sinus venosus through a number of interlacing muscle fibres for conveying the impulses for contraction to this chamber. On the right side in the enclosure, formed by the invagination of the lateral portion of the sinus venosus, is present an area of mesh-work of nerve and muscle fibres which may be stated as the nodal area. It resembles the S-A node in the structure and the general disposition of its fibres and nuclei.

Atria: The atria of *Gallus* correspond very closely in form and disposition with those of other birds. The muscles (musculi pectinati) of the atria are thick bundle-like structures (connected together through elongated fibrous bands) running beneath the endocardium. This type of musculature is in correlation with the small size of the atria as it gives them, during atrial systole, enough force to propel the blood forward from the atria into the ventricle.

Atrioventricular node: The A-V node is not so clearly defined as the S-A node. The A-V node is present on the left side of the interatrial septum at the base of the aorta. It is a clear zone of numerous distinct, large cells with prominent nuclei. The nuclei of the node are larger than those of the A-V bundle and they are equally distributed in every portion of the node. The A-V node is not bounded by any fibrous sheath, but there appear in cross-sections longitudinal fibres on either side of it to give it a definite shape. A thick band of 'Purkinje fibres' extends from the caudal end of the node to connect it with the base of the interatrial septum. The 'Purkinje fibres' as distinguished from other fibres in the present investigation have the microscopic characteristics (Kistin, 1949) of the fibres first observed by Purkinje (1845) in sheep and other mammals. These fibres are larger, have few smaller muscle fibres, and they cross each other so as to look like cells in transverse sections. The A-V node extends caudally towards the A-V bundle and continues into the ramifications of 'Purkinje fibres' of the A-V bundle. The cells, the nuclei and the fibres of the node take a very deep stain with acid fuchsin and are, therefore, differentiated easily from other cardiac fibres.

Atrioventricular bundle: The A-V bundle (bundle of His) is present in the cephalic portion of the ventricular septum near the base of the right A-V valve. It is ventral and caudal in position to the A-V node. It extends deep into the ventricular septum and then bifurcates into a right and a left limb. The right limb proceeds towards the right muscular valve, while the left limb takes a somewhat curved course through the myocardial fibres and extends along the left lateral border of the ventricular septum. The structure of the fibres and the cells of the A-V bundle resembles those of the A-V node.

The muscular right atrioventricular valve has the same structure as that described by Davies (1930) in other birds.

Purkinje fibres: The presence of these pale, multinucleated fibres has long been noted and their distribution has been extensively studied in birds (Davies, 1930; Adams, 1937). The system of these fibres in *Gallus* is not so well developed as in other birds. The atria are devoid of such fibres. However, small columns of 'Purkinje fibres' are present surrounding the atrioventricular orifices. These columns appear as rounded bundles in transverse sections (Fig. 4). There are three such bundles of 'Purkinje fibres' present round the right atrioventricular orifice and two round the left atrioventricular orifice. A band of 'Purkinje fibres' extends also along the left lateral border of the right atrioventricular orifice and sends an early branch to the caudal portion of the A-V node. 'Purkinje fibres' which cross each other look like cells. The cells thus formed are not of uniform shape. Some are spherical, some elliptical and others elongated. Generally, there is one nucleus in each cell, but some have two, and there are others which have no definite nucleus. The cells without nuclei are mostly on the periphery and not in the centre.

DISCUSSION

The presence of a ridge inside the sinus venosus of the heart of the Indian fowl assumes special significance as it has also been observed in a number of reptiles. O'Donoghue (1920) described a tuberculum intervenosum separating the aperture of the left precaval vein from that of the postcaval vein in *Sphenodon punctatus*. Goodrich (1930) observed a ridge (septum sinu venosi) between the openings of the left precaval and postcaval veins on one side and that of the right precaval vein on the other. Mathur (1946) found a prominent ridge in *Natrix piscator* separating the left precaval and the postcaval. In *Gallus* the ridge has come further down very near to the base of the sinuatrial valves. Its disposition indicates that the two precavals and the postcaval open not only separately but also directly (particularly the postcaval) into the right atrium. This shows that the heart of this bird makes an approach towards a condition found in the hearts of other birds and



FIG. 1. Longitudinal section through the sinuatrial node. $\times 700$.

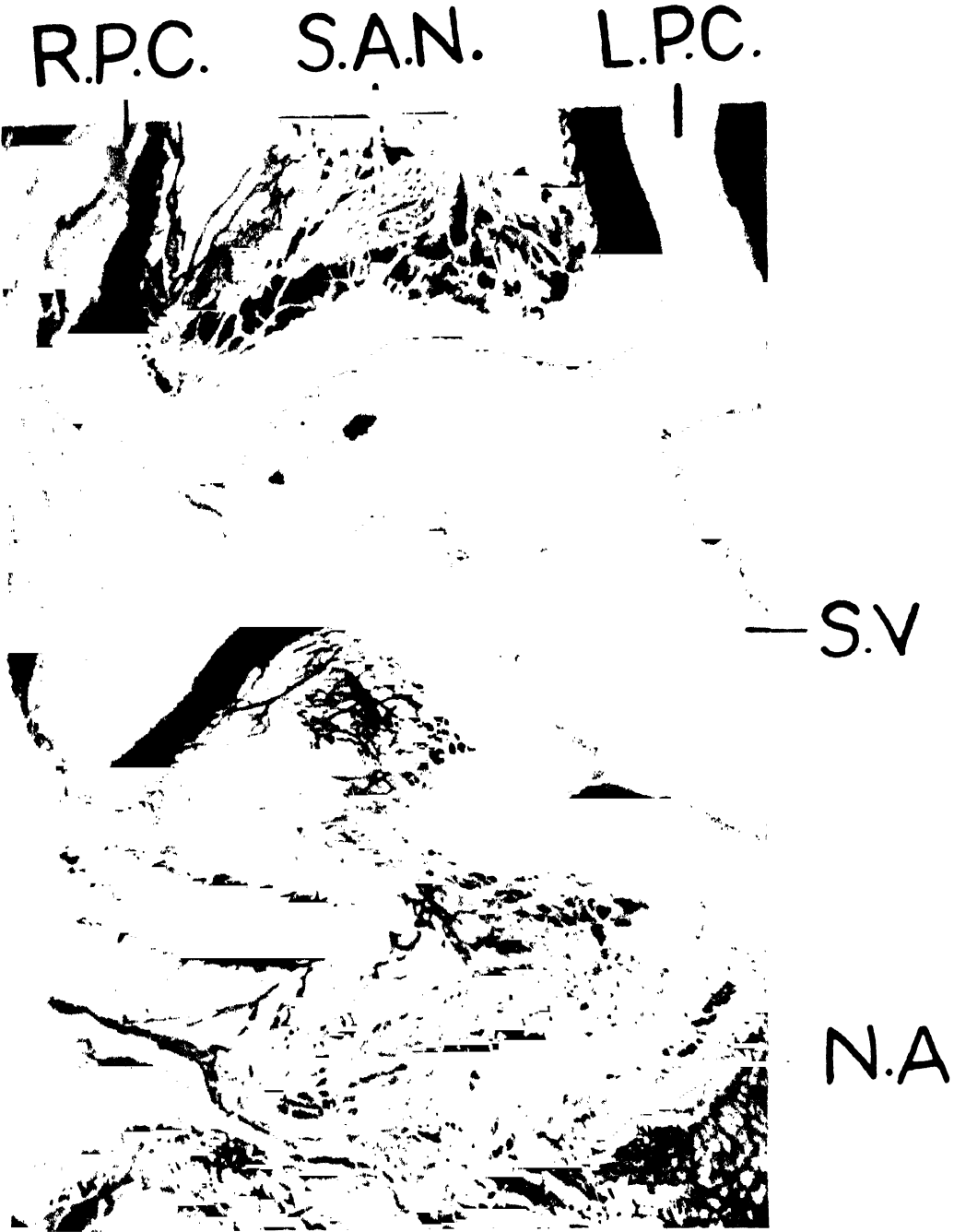


FIG. 2. Longitudinal section through the cephalic portion of sinus venosus.

mammals where the sinus venosus is absent and the three caval veins open separately and directly into the right atrium.

The sinus venosus, a prominent chamber in the heart of lower vertebrates, disappears in many birds and mammals. The disposition of sinus venosus in *Gallus* presents interesting transitional stages in the formation of the mammalian S-A node in relation with and consequent on the reduction of sinus venosus. An invagination in the right wall of the sinus venosus has been observed for the first time in *Gallus*. It marks the beginning of a process by which this chamber is ultimately lost in birds and mammals. It is easy to postulate, then, that a further inpushing in this wall of the sinus venosus will result in the separation of the major cephalic portion from a very small caudal portion. The loss of the cephalic portion means the disappearance of the entire functional sinus venosus, because the caudal part only acts as the *via media* to convey the venous blood immediately into the right atrium. With the reduction of the sinus venosus, the nodal area and the S-A node which are present on either side of this chamber will get united to form an extensive S-A node extending from the base of the right sinuatrial valve over to the cephalic and dorsal side of the right atrium. Such a postulation is important as it throws light on the phylogeny of the S-A node. It is to be remembered that in many birds and mammals (Davies, 1930; 1931) the S-A node has an extensive disposition as the sinus venosus is absent.

The absence of sinus venosus in birds and mammals results in the reduction of the sinuatrial ring also. Walmsley and Mackenzie (cited by Davies, 1931) stated that the reduction of the S-A ring is due to the fusion of the left sinus valve with the interatrial septum. In *Gallus* the presence of the left sinuatrial valve which has not fused with the interatrial septum, the presence of the S-A node and the reduced nature of the sinus venosus, indicate that the reduction of the S-A ring is not due to the fusion of the left sinus valve but is due to the reduced nature of the sinus venosus.

Kishne and Musha (1952) observed from a study of electrocardiograms that, in a case of partial sinuatrial block, the sinus impulses are not conducted to the atria but directly to A-V node. From the A-V node these impulses are transmitted to the ventricles on the one hand and to both the atria on the other. These workers expressed the view that the sinus impulses do not travel radially in all directions over the atria but are conducted through specific pathways. The serial sections through the sinuatrial junction of the heart of *Gallus* revealed that the impulses initiated at the S-A node can be transmitted from the node to the sinus venosus through the interlacing fibres connecting the former with the latter. From the sinus venosus the impulses can spread over to the right atrium through the right sinuatrial valve which offers the necessary muscular continuity for the transmission of these impulses from the sinus venosus to the right atrium. The interatrial septum may also receive the sinus impulses by way of the left sinuatrial valve which connects the sinus wall, on the left side, with the cephalic part of the atrial septum. From the interatrial septum, the impulses cannot reach the A-V node directly but through a connecting band of special fibres present between the caudal portions of the septum and the A-V node. Such a continuity in the path for the wave of contraction indicates that the impulses may travel along set routes as stated by Kishne and Musha (1952).

Dr. Kistin (1953) in a private communication to the author wrote: 'When I read Purkinje's paper I was concerned with the meaning of the term "Bewegungsapparat", and received little help from people who know German well. One suggestion was supporting (skeletal) structure for the cardiac muscle. Certainly Purkinje's paper does not suggest any conducting function to these fibres'. Davies and Francis (1946) believe that 'Purkinje fibres' constitute special movement apparatus (Bewegungsapparat) and are part of special conducting tissue. In the heart of *Gallus* a special band of Purkinje fibres has been observed to connect the

interatrial septum and the A-V node and it may be said that the impulses received by the septum are conveyed through these fibres to the A-V node. The A-V bundle which affords the only connexion for the transmission of the impulse from the atria to the ventricles also consists of Purkinje fibres. These facts strongly suggest that Purkinje fibres can be regarded as conducting fibres.

SUMMARY

1. Sinuatrial node, atrioventricular node, atrioventricular bundle and Purkinje fibres are present in the heart of the common Indian fowl for initiating and conducting the cardiac rhythm of contraction.

2. The invagination in the lateral wall of the sinus venosus in *Gallus* and the presence of a ridge inside this chamber mark the beginning of a process by which the sinus venosus gradually disappears in birds and mammals so that the three caval veins open separately and directly into the right atrium.

3. The sinuatrial node of birds and mammals develops in relation with and consequent on the reduction of the sinus venosus.

4. The atrioventricular node should not be regarded as a derivative of the sinuatrial ring of the lower vertebrate heart (fish, amphibia and reptile).

5. It is suggested that the impulses initiated at the sinuatrial node travel along set routes from one chamber of the heart to the other and the atrioventricular node cannot receive the sinus impulses directly from the sinuatrial node but only through the sinus venosus, the left sinuatrial valve, the interatrial septum and the connecting band of special fibres.

6. The probable path which the cardiac impulse may follow from the S-A node to the A-V node has been described.

ACKNOWLEDGEMENT

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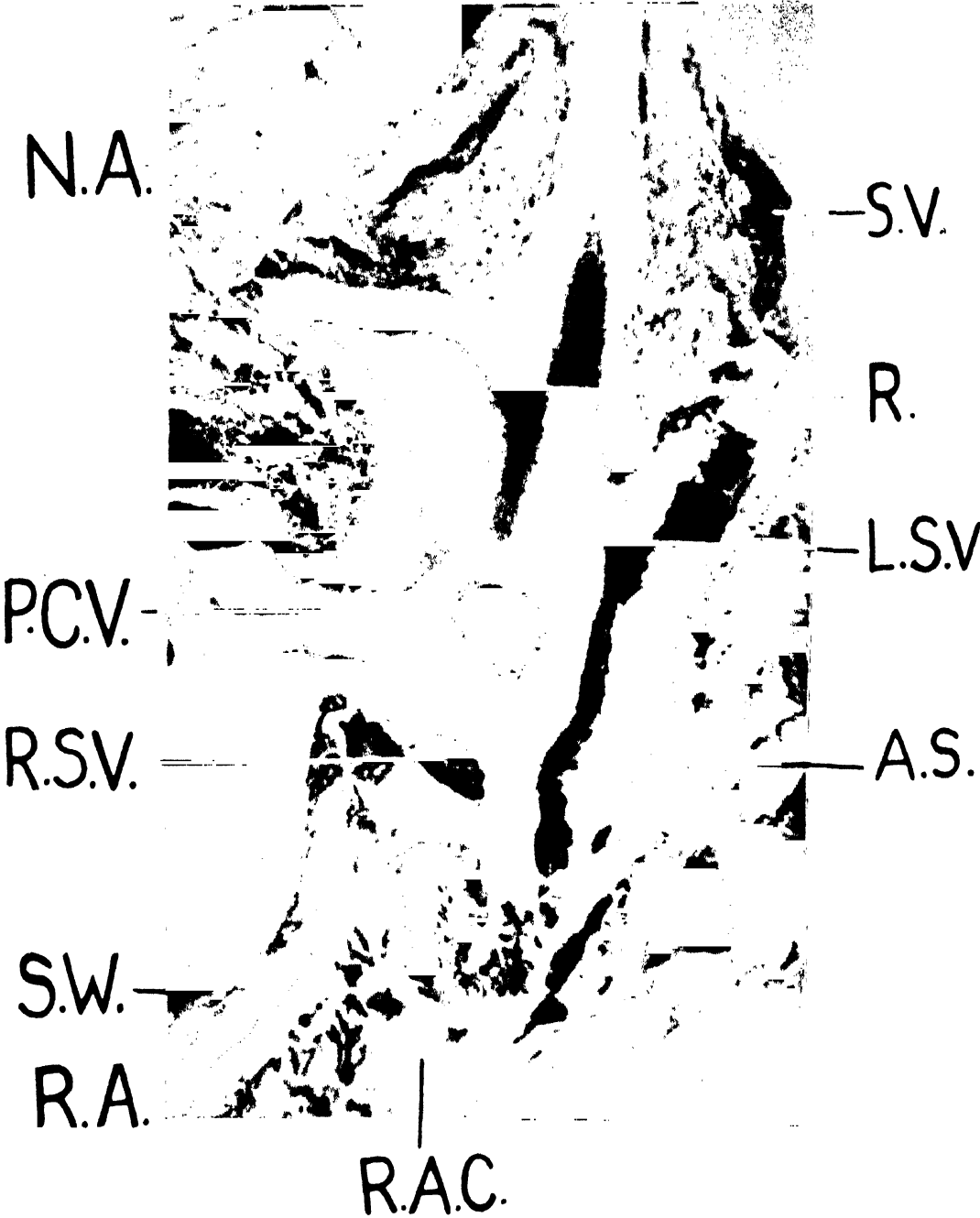


Fig. 3. Longitudinal section through the caudal portion of sinus venosus. $\times 300$.

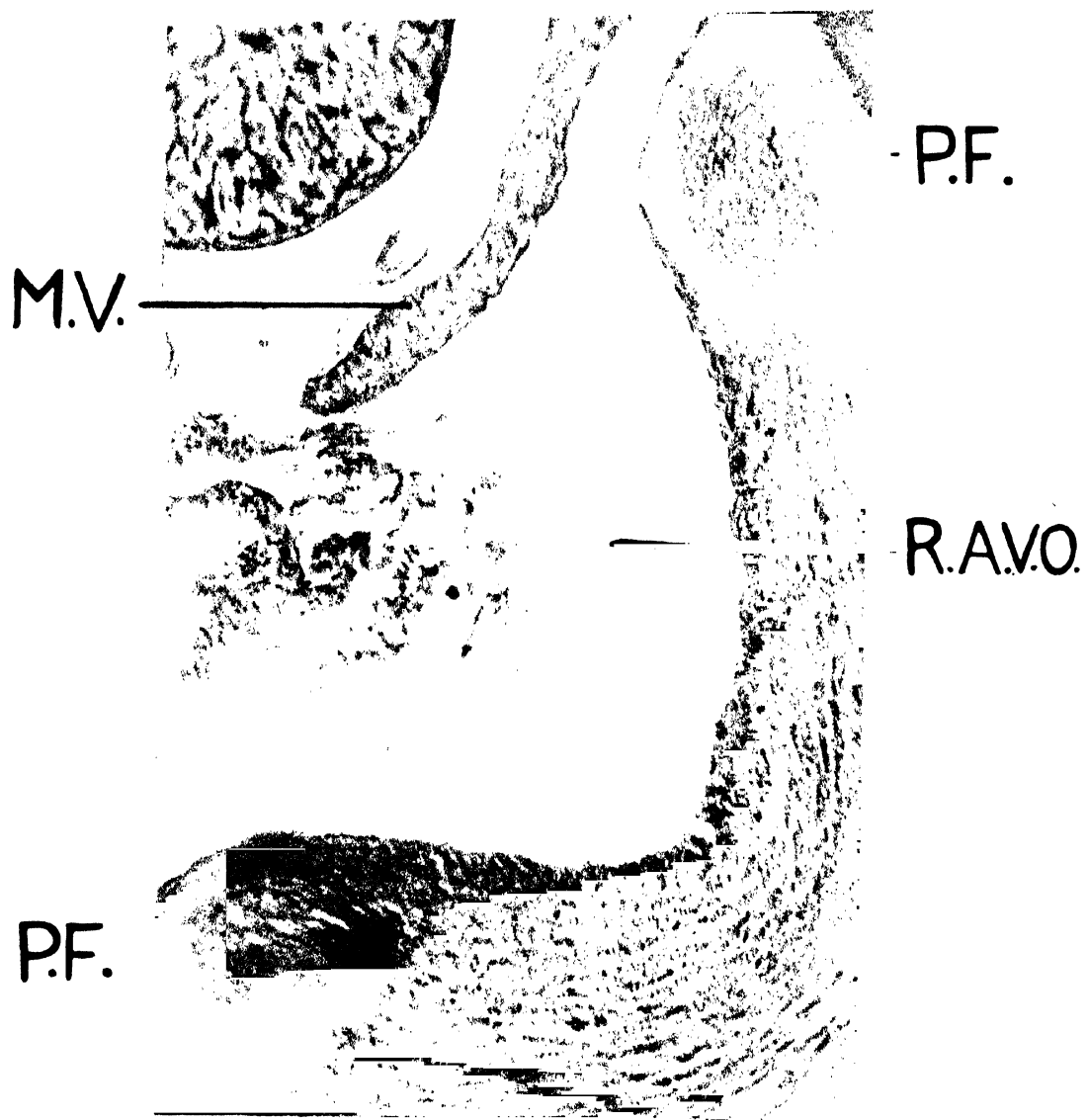


FIG. 4. Transverse section through the junction of the right atrium and the ventricle to show the right atrioventricular ring of Purkinje fibres. 225.

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ABBREVIATIONS

A.S.—Atrial septum; L.P.C.—Left precaval vein; L.S.V.—Left sinuatrial valve; M.V.—Muscular right atrioventricular valve; N.A.—Nodal area; P.C.V.—Postcaval vein; P.F.—Purkinje fibres; R.—Ridge; R.A.—Right atrial wall; R.A.C.—Right atrial cavity; R.A.V.O.—Right atrioventricular orifice; R.P.C.—Right precaval vein; R.S.V.—Right sinuatrial valve; S.A.N.—Sinuatrial node; S.V.—Sinus venosus; S.W.—Sinus wall.

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**MORPHOLOGY AND HISTOLOGY OF THE AIR-
BLADDER OF *HILSA ILISHA* (HAMILTON) AND
GADUSIA CHAPRA (HAMILTON) AND THEIR CONNEC-
TION WITH THE INTERNAL EAR ***

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(Communicated by D. R. Bhattacharya, F.N.I.)

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INTRODUCTION

A number of workers have studied the air-bladder of American and British clupeoid fishes. Hasse (1873) is one of the earliest workers in this field and he investigated the ear and the air-bladder diverticulum in *Clupea alosa* and *C. harengus*. Retzius' (1881) description of the air-bladder of *C. harengus* agrees essentially with that of Hasse. Mathews (1886), in an investigation of the skeleton of British clupeoid fishes, has described the bony canals and capsules in the herring (*C. harengus*), the pilchard (*C. pilchardus*) and the shad (*C. alosa*). Later, Ridewood (1891) described the ear-air-bladder relation in the British species, viz. herring, pilchard, shad, sprat, thwait and anchovy. Tracy (1920) worked out the air-bladder of different American clupeoids. But, so far, no work has been attempted on the air-bladder of clupeoids in India and I am very much thankful to Dr. Bhattacharya for suggesting this problem to me. Strangely enough even the workers mentioned above have concentrated their attention only on the ear-air-bladder connection and have left out the histological work which, in the present case, has revealed many features of considerable interest.

I am indebted to Dr. D. R. Bhattacharya, formerly Vice-Chancellor of the University of Allahabad, for his continuous guidance, help and encouragement. I am also grateful to the late Dr. S. L. Hora, Director, Zoological Survey of India, for going through the manuscript. The present work was carried out during the tenure of a Senior Research Fellowship awarded by the Government of India to which I wish to offer my thanks.

MATERIAL AND TECHNIQUE

Both *Hilsa ilisha* and *Gadusia chapra* are available at Allahabad all the year round, but are found in abundance from August to November. The fishes were opened along the mid-ventral line. To fix the lining epithelium of the air-bladder in distended condition, it was necessary to puncture the bladder posteriorly (*in situ*) and immediately filling it with the fixative used, and also pouring the fixative over the bladder. By doing this all the shrinkage of the bladder was avoided on removal from the body. The chief fixative generally used was Bouin's picro-formol-acetic fluid. Sections of 6μ to 8μ were cut and stained with Delafield's haematoxylin and counterstained with 0.5%-1.0% alcoholic eosin. The bony matter was decalcified before embedding by a 3% solution of nitric acid in 70% alcohol, and changing it every alternate day. Care was, however, taken to avoid over-decalcification.

* Part of the thesis accepted for the degree of Doctor of Philosophy in Science in the University of Allahabad, India.

OBSERVATIONS

Hilsa ilisha

(a) *External morphology*: The air-bladder in *H. ilisha* is elongated and extends the whole length of post-pericardiac coelom (Pl. V, Fig. 1, A.B.). On the ventral side it is intimately invested with the peritoneal lining, whereas dorsally it is in contact with the body-wall and separated from the vertebral column by the aorta and the kidney. Its wall is smooth, thick and appears brownish in collapsed condition and shining when inflated. The diameter of the air-bladder is not uniform, the width being greatest in the middle, which is more distensible than the two extremities. The bladder suddenly tapers posteriorly and ends blindly in the vicinity of the anal opening. In this case the bladder does not open to the exterior as observed in certain American and British clupeoid fishes. Towards the anterior side also, the bladder narrows down gradually and becomes cylindrical until, in the region of heart, it divides into two diverticulae of extremely thin calibre. These are called the air-ducts (Pl. V, Fig. 1, AD).

The ductus pneumaticus arises from the posterior blind end of the cardiac portion of the stomach and opens into the air-bladder in the mid-ventral median line (Pl. V, Fig. 1, Pn. D.). The diameter of the pneumatic duct is greater near the stomach and comparatively less at the air-bladder end. It is an open communication between the air-bladder and the stomach, and it usually contains some mucus. The opening of the pneumatic duct in the stomach is guarded neither by valves nor by sphincter muscles.

(b) *Histology*: The air-bladder wall of *H. ilisha* consists of two layers—tunica externa and tunica interna (Pl. VI, Fig. 1, Tu. ex, Tu. in). The former is composed mainly of two layers, i.e. an external special 'nacreous layer' which is very thin and an inner muscular (and not elastic fibres) layer which forms the major part of the tunica externa. The tunica interna consists of a layer of conjunctiva tissue which is not very thick and an innermost layer of cells which have become larger and columnar. Masses of blood capillaries are packed together in these cells and are covered over by unmodified internal epithelium of the air-bladder (Pl. VI, Fig. 1). The precoelomic diverticulum of the air-bladder is simply the tubular prolongation of the air-bladder and consists of both tunica externa and tunica interna.

The wall of the ductus pneumaticus (Pl. VI, Fig. 3) shows the same layers as those of the air-bladder, but it differs from the latter in the absence of the closely aggregated blood capillaries. The other very interesting feature, hitherto unreported, is the division of the ductus pneumaticus cavity. This is brought about by the formation of two septa in the inner wall of the pneumatic duct which go on developing towards each other until they meet in the middle and fuse completely dividing the cavity into two (Pl. VI, Figs. 4-6). In due course one of the chambers enlarges, the other one consequently diminishes gradually and ultimately disappears, and a single cavity is left. This happens at interval at several places throughout the length of the pneumatic duct.

(c) *The precoelomic diverticulum*: As the air-bladder passes anteriorly over the pericardial cavity, it comes to lie between the head kidneys of the two sides. The intercostal aponeurosis, in this region, sends off two layers of considerable strength, one running dorsal and the other ventral to the diverticulum. The precoelomic diverticulum (Pl. V, Fig. 1, Pr. D.), supported by the aponeurotic tubular sheath, runs anteriorly and becomes enclosed in a cartilaginous canal. The diverticulum being very delicate and narrow in calibre, the aponeurotic sheath and the 'cartilage canal' appear to give protection to it. The 'cartilage canal' is formed gradually round the diverticulum and not abruptly as in several species of *Clupea*.

(d) *The air-ducts*: The cartilage tube of the precoelomic diverticulum forms a Y-shaped structure, the base of which is single, median and short. Anteriorly, just behind the dorsal aorta (Pl. V, Figs. 1 and 2), it is divided into the two arms of

a Y which are called the air-ducts. These ducts are also formed of both tunica externa and tunica interna and are surrounded by a protective cartilage canal (Pl. VI, Fig. 2).

The air-ducts of each side pass forwards and slightly outwards and upwards, and after running for a short distance in a groove in the exoccipital bone, penetrate the latter at a point situated immediately behind the fenestra between the exoccipital and the basioccipital, known as the auditory fenestra.

(e) *The air-vesicles and ear-air-bladder connection* : The air-ducts of each side, after penetrating the exoccipital bone, dilate to form a fusiform chamber (Pl. V, Fig. 2). The wall of the chamber is thin, membranous and silvery white in appearance. The lumen of the air-ducts, though very fine, is continuous with that of the fusiform chamber.

At the point where the exoccipital, prootic and pterotic bones meet, the anterior narrow end of the fusiform chamber gives off a lateral branch which enters a spherical cavity in the pterotic bone known as the posterior or pterotic air-vesicle (Pl. V, Fig. 2, PAV). The main branch of the fusiform chamber continues forward in a horizontal direction in a canal in the prootic bone, where it ends in a dilated vesicle known as the anterior or prootic air-vesicle (Pl. V, Fig. 2, AAV).

The anterior air-vesicle is nearly spherical in shape and is flattened on the upper side where a blind caecal prolongation of the recessus utriculi of the membranous vestibule of the ear touches it through the opening situated dorso-posteriorly in the prootic bony capsule (Pl. V, Fig. 2). It is of interest to note that the flattened dorsal wall of the anterior air-vesicle after uniting with the flat side of the blind caecum acts as tympanic membrane and thus the ear-air-bladder connection becomes very intimate.

The posterior air-vesicle is comparatively smaller than the anterior one and is not in any direct connection with the membranous labyrinth, but it lies within the loops of the horizontal semi-circular canal.

Gadusia chapra

The air-bladder in the case of *G. chapra*, except for a few minor differences, resembles that of *H. ilisha* in both morphology and histology. The air-bladder (Pl. V, Fig. 3, A.B.) is tubular and ends posteriorly in the vicinity of the anus without opening outside. Anteriorly, this is continued into the precoelomic diverticulum and then gets enclosed in the cartilage tube. The diverticulum divides into two air-ducts which penetrate the exoccipital bone and swells up into a fusiform chamber. It later on divides into the anterior and posterior air-vesicles (Pl. V, Fig. 3, AAV, PAV).

The pneumatic duct is large and is an open connection between the air-bladder and the alimentary canal. As in *H. ilisha*, the pneumatic duct becomes divided into two chambers at several places (Pl. VI, Fig. 8).

The bladder-wall (Pl. VI, Fig. 7) is thin and is composed of tunica externa and tunica interna. The muscular layer is not so well developed as in *H. ilisha*. Nacreous layer is found to be absent in this case. The innermost layer of tunica interna is not modified into columnar cells, but the blood capillaries are present, though not so much aggregated as in *H. ilisha*.

DISCUSSION

The histology of both the air-bladder and the pneumatic duct of *H. ilisha* and *G. chapra* has revealed many interesting features. Corning (1888) and Eissele (1922) have described that the air-bladder of trout possesses longitudinal and circular muscle fibres. Evans (1925) has given a detailed account of the intrinsic musculature in cyprinids. But the muscular layer in *H. ilisha* is very much enormously developed and, as far as the author is aware, such a well developed muscular layer has not

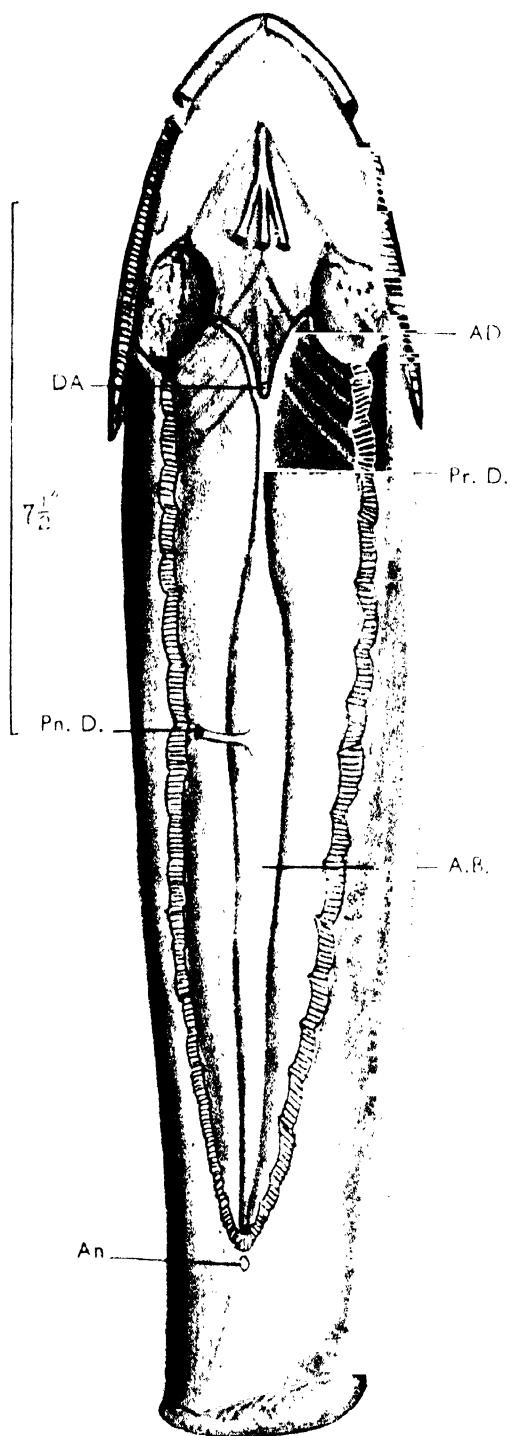


Fig. 1

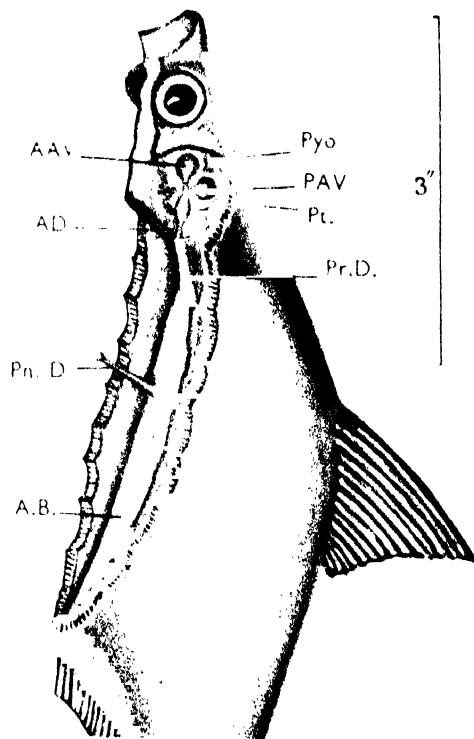


Fig. 3

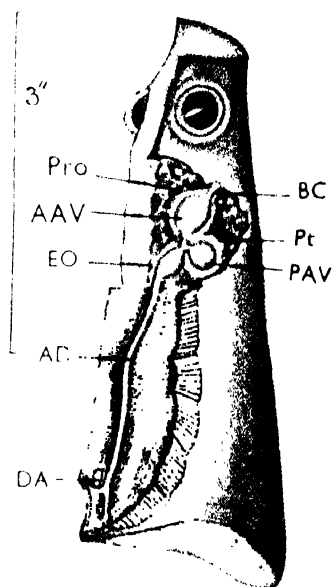


Fig. 2

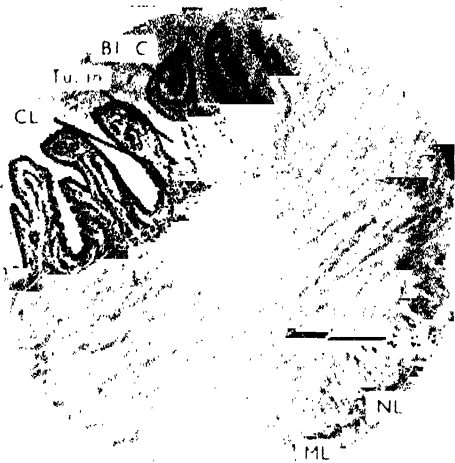


Fig. 1

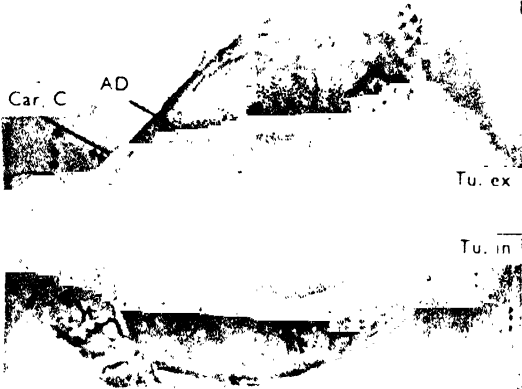


Fig. 2

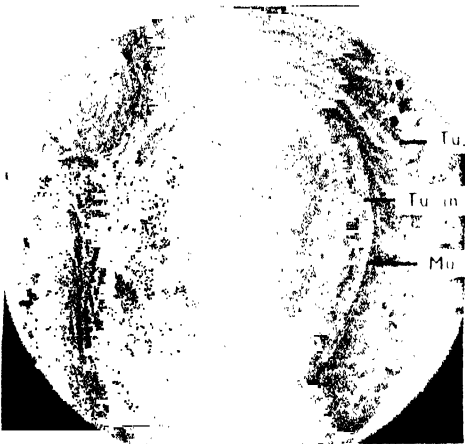


Fig. 3



Fig. 4



Fig. 5

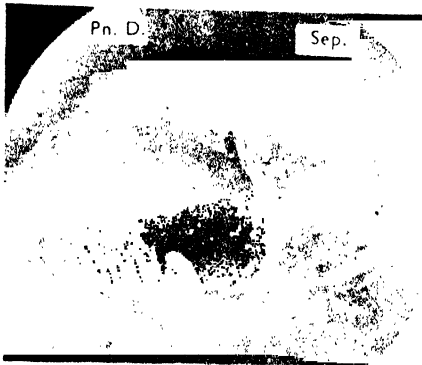


Fig. 6

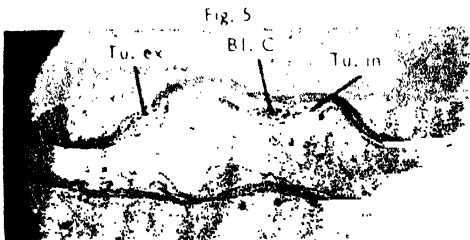


Fig. 7



Fig. 8

yet been recorded in any other physostomous fish. *G. chapra* also possesses this muscular layer, but it is not so well developed.

Corning (1888), de Beaufort (1909), Rauther (1922) and Maier and Scheuring (1923) have described that, among Clupeidae and Salmonidae, the inner lining of the air-bladder is thickened into cylindrical epithelium. The numerous capillaries which supply this epithelium do not merge to form the retia mirabilia. However, Rauther describes and figures associations of blood vessels in *Cyprinus carpio* and *Carassius carassius*, and he calls them retia mirabilia. Vincent and Barnes (1896) have grouped the red-bodies into two groups. In the first group, the red-bodies may properly be called red glands, because in these cases the lining epithelium of the air-bladder passes over the masses of blood capillaries and becomes modified to form a distinctly glandular epithelium. Woodland (1911) has shown that, in these cases, the masses of blood capillaries get arranged and are known as retia mirabilia which may or may not be in communication with the glandular epithelium. Such types of glands are met with in physoclistous fishes. The second group, as described by Vincent and Barnes (1896), is represented by *Anguilla anguilla* where the red-bodies consist of simply a mass of capillary blood-vessels covered by an unmodified continuation of the internal epithelium of the air-bladder. In such types of fishes there is no true gland. In *H. ilisha*, the innermost layer of tunica interna has become large and columnar. Masses of blood capillaries are found here and they are covered over by an unmodified internal epithelium. I agree with Vincent and Barnes in that only such structures should be referred to as 'red-bodies' or 'vascular bodies'. In *G. chapra* the concentrations of blood capillaries are not found to that extent as in *H. ilisha*.

The volume of the air-bladder in physostomous fishes is controlled by swallowing air at the surface or releasing gas bubbles. The gas enters or leaves the air-bladder through the pneumatic duct. In *H. ilisha* and *G. chapra*, the pneumatic duct is a relatively long, thick-walled tube running from the air-bladder and opening into the blind sac of the stomach. Generally this opening is guarded either by a valvular arrangement or by sphincter muscles. Both these devices control the passage of air from the air-bladder to the alimentary canal and vice versa. But in *H. ilisha* and *G. chapra* none of these mechanisms are present, but instead a very unique and interesting development has come into existence. This has been brought about by the formation of two septa in the inner wall of the pneumatic duct which go on developing towards each other till they meet and fuse, and thus completely divide the cavity into two. Later on, one of the chamber enlarges at the expense of the other, which consequently diminishes in size and ultimately disappears. Such a division occurs at several places in the length of the pneumatic duct. As a result of this arrangement, the air does not find an easy passage either from the stomach to the air-bladder or in the opposite direction, because it has to pass through a circuitous route caused by growths inside the pneumatic duct. But still the pneumatic duct does not function as an automatic safety valve which is forced open when the pressure within the air-bladder rises to a critical level. This remarkable mechanism which has taken up the function of the valvular arrangement or the sphincter muscles has not yet been recorded in any other fish. It is worth while examining if such an arrangement is also present in the British and American clupeoid fishes.

The ear-air-bladder connection essentially resembles that of the American and British clupeoid fishes with some minor differences, e.g. the precoelomic diverticulum and the air-ducts in *H. ilisha* and *G. chapra* consist of both the tunica externa and the tunica interna and not the latter only as stated by Tracy (1920) in the American clupeoid fishes.

SUMMARY

The air-bladder in *H. ilisha* is elongated and extend the whole length of post-pericardiac coelom and is intimately invested with the peritoneal lining ventrally. The air-bladder ends blindly in the vicinity of the anal opening. The wall of the air-bladder consists of the tunica

externa, with a nacreous layer and a very well developed 'muscular layer; and the tunica interna where the masses of blood capillaries are packed together is covered over by an unmodified lining epithelium. This structure should be regarded as 'red or vascular bodies'. There is no valvular arrangement or sphincter muscles at the opening of the pneumatic duct in stomach, but its function is performed by the divisions of pneumatic duct at several places. The ear-air-bladder connection is essentially similar to that of other American and British clupeoid fishes.

The air-bladder of *G. chapra* resembles with *H. ilisha* in all details, except that in the former the muscular layer in the tunica externa and the 'red-bodies' in the tunica interna are not so well developed.

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EXPLANATION OF PLATES

PLATE V

- Fig. 1. *H. ilisha*—Dissected ventrally to show the air-bladder. Pneumatic duct has been cut.
- „ 2. *H. ilisha*—Head dissected latero-ventrally to show the air-ducts ending in the anterior and posterior air-vesicles.
- „ 3. *G. chapra*—Dissected ventro-laterally to show the disposition of air-bladder. Pneumatic duct has been cut. Air-ducts and air-vesicles are also shown.

PLATE VI

- Fig. 1. *H. ilisha*—Photomicrograph of T.S. of the air-bladder showing tunica externa and tunica interna. Innermost columnar layer is packed with blood capillaries. Tunica externa has well developed muscular layer.
- „ 2. *H. ilisha*—Photomicrograph of T.S. of the air-duct showing the cartilage canal, tunica externa and tunica interna.
- „ 3. *H. ilisha*—Photomicrograph of T.S. of the pneumatic duct showing the usual layers of the air-bladder wall.
- Figs. 4-6. *H. ilisha*—Photomicrographs of T.S. of the pneumatic duct showing its division.
- Fig. 7. *G. chapra*—Photomicrograph of T.S. of the air-bladder wall showing tunica externa, tunica interna and blood capillaries.
- „ 8. *G. chapra*—Photomicrograph of T.S. showing the division of the pneumatic duct.

ABBREVIATIONS

<i>AAV</i>	..	Anterior air-vesicle.
<i>A.B.</i>	..	Air-bladder.
<i>AD</i>	..	Air-duct.
<i>AN</i>	..	Anal opening.
<i>BC</i>	..	Blind caecum of recessus utriculi.
<i>Bl. C.</i>	..	Blood capillaries.
<i>CL</i>	..	Columnar layer.
<i>Car. C</i>	..	Cartilage canal.
<i>DA</i>	..	Dorsal aorta.
<i>EO</i>	..	Exoccipital bone.
<i>ML</i>	..	Muscular layer.
<i>Mu</i>	..	Mucus.
<i>NL</i>	..	Nacreous layer.
<i>PAV</i>	..	Posterior air-vesicle.
<i>Pn. D.</i>	..	Pneumatic duct.
<i>Pr. D.</i>	..	Precoelomic diverticulum.
<i>Pro, Pyo</i>	..	Prootic.
<i>Pt</i>	..	Pterotic.
<i>Sep.</i>	..	Septa dividing the pneumatic duct.
<i>Tu. ex</i>	..	Tunica externa.
<i>Tu. in</i>	..	Tunica interna.

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ON THE MORPHOGENESIS OF THE YOLK-SAC GLAND IN CHIROPTERA

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1. INTRODUCTION

As early as 1840, Owen described the foetus of 'Rousette' and drew attention to the strange appearance of the persisting umbilical vesicle constituting a kidney-shaped mass with folds on its surface. The diagram of Robin (1881) of the gravid uterus nearly at full term of *Pteropus edwardsii* shows the presence of a gland near the placenta.

Branca (1923) stated that the yolk-sac was converted into a gland of internal secretion. Göhre (1892) found that the yolk-sac of *Pteropus edulis* assumed the form of a coiled gland-like organ. Sprengel (1932) described the persistence of the yolk-sac in Megachiroptera in the form of a gland of internal secretion. No reasons were given by him or by Branca for attributing an endocrine function to this gland. Moghe (1951) also observed the conversion of the yolk-sac into a gland-like organ in *Pteropus giganteus giganteus*. He gave a photomicrograph of the structure of the gland nearly at full term.

Mossman (1937) in the table giving the details of implantation, amniogenesis and foetal membranes of mammals stated that in Megachiroptera (*Pteropus edulis* and *Xantharpya amplexicaudata*) 'the lumen of the yolk-sac in later stages of gestation is obliterated and the walls come close together and a solid vascular body is formed'. In the details given in the tables for the suborder Microchiroptera (families: Rhinolophidae, Hipposideridae, Phyllostomatidae, Vespertilionidae and Molossidae), no mention is made of the conversion of the yolk-sac into a gland in any of the species belonging to these families of Microchiroptera. Judging from the detailed accounts of the development of these species of Microchiroptera it is certain that in none of them the yolk-sac is converted into a gland. This appears to be one of the differences between the Mega- and Microchiroptera.

Since the publication of Mossman's paper, the embryology of some more species of Microchiroptera, e.g. *Artibeus jamaicensis pervipes* (Wislocki and Fawcett, 1941), *Myotis lucifugus lucifugus* (Wimsatt, 1945), *Scotophilus wroughtoni* (Gopalkrishna, 1949 and 1950), *Lyroderma lyra lyra* (Gopalkrishna, 1950), *Taphozous longimanus* (Gopalkrishna, unpublished) and *Rhinopoma kinneari* (Srivastava, 1952), has been described. Only in two of these, namely *Taphozous longimanus* (Fam.: Emballonuridae) and *Rhinopoma kinneari* (Fam.: Rhinopomidae), it has been found that the yolk-sac is converted into a gland-like structure as it is in Megachiroptera (Moghe, 1954).

It would be of interest to record here that the site of the chorio-allantoic placenta is lateral in *Taphozous longimanus* and mesometrial in *Rhinopoma kinneari* as in Megachiroptera though in the other species of Microchiroptera it is antimesometrial.

The Microchiroptera consist of 17 families (Simpson, 1945). It would be of some interest if characters are found in the two families, viz. Emballonuridae and Rhinopomidae, and probably in some others whose embryology is not yet known, which would connect the Mega- with the Microchiroptera.

It is also interesting to note that Chiroptera is the only order amongst the mammals in which some of the species show the conversion of the yolk-sac into a gland-like structure. Its presence in only some members of only one group of mammals is difficult to explain. But it is possible to ascertain the nature and function of this gland. It is from this point of view that a detailed study of the yolk-sac has been undertaken.

2. MATERIAL AND METHODS

Gravid uteri of *Pteropus giganteus giganteus*, *Cynopterus sphinx gangeticus* and *Taphozous longimanus* were collected round about Nagpur (Madhya Pradesh), and the uteri in various stages of pregnancy were sectioned. Sections were prepared by the usual paraffin method. Yolk-sac glands were also removed from the uteri showing very advanced pregnancy and they were fixed and sectioned.

I have in my possession very nearly complete series of uteri of these three species so that progressive changes in the yolk-sac could be ascertained. I had the additional advantage of examining sections of the gravid uteri of *Pteropus* and *Cynopterus* from the collection of Prof. Moghe and of *Taphozous longimanus* from the collection of Mr. A. Gopalkrishna. *Rhinopoma kinneari* was not available in Nagpur, but Dr. S. C. Srivastava sent me gravid uteri of this species fixed in various fixatives from Gwalior where they are found abundantly. I have also examined the sections of the gravid uteri of this species in the collection of Mr. A. Gopalkrishna.

3. OBSERVATIONS

(a) *Pteropus giganteus giganteus* (Brünnich):

General history of the yolk-sac.—The early formation of the yolk-sac and the subsequent changes it undergoes have been described by Moghe (1951). In the late limb-bud stage, the yolk-sac commences to assume the shape of a shrivelled bag. The entire yolk-sac lies partly on the lateral and partly on the mesometrial side below the chorio-allantoic placenta. In a slightly more advanced stage the wall of the yolk-sac is thrown into folds and its lumen is considerably obliterated. Thus the yolk-sac becomes a bag with a very narrow lumen (Pl. VII, Fig. 1). At mid-term, the walls approximate more closely and the original lumen of the yolk-sac is obliterated except for narrow slit-like spaces. It is very much reduced in size and lies completely below the chorio-allantoic placenta. During advanced pregnancy, it is converted into a gland-like structure (Pl. VII, Fig. 2, and Pl. VIII, Fig. 9).

Histological changes in the yolk-sac wall.—In the early limb-bud stage the yolk-sac wall is not uniform in thickness. The abembryonic wall of the yolk-sac is thinner. The endoderm cells are columnar and have prominent nuclei and the cells lie close together (Pl. VII, Fig. 3). On the mesodermal side the endoderm cells have large vacuoles. Mesoderm cells are fusiform and lie scattered. On the lateral side the wall of the yolk-sac is comparatively thick (Pl. VII, Fig. 4). Between the endoderm and mesoderm layers vitelline vessels with nucleated corpuscles are present (Pl. VII, Fig. 4). These give a beaded appearance to the yolk-sac wall. At the limb-bud stage, the entire yolk-sac wall is thrown into folds. Any portion of the wall shows essentially the same type of folding. The endoderm cells fold to form villi-like outgrowths each enclosing a space. Thus tubules are formed (Pl. VII, Fig. 5), each with a lumen, and the endoderm cells forming the tubule are large with prominent nuclei. The mesoderm cells retain the original position, but form masses over the endoderm wall and its villi. The villi of the endoderm sink deeper and separate from the rest, but the endoderm of the wall folds to form additional tubules (Pl. VII, Fig. 6), the spaces within them representing the ducts of the tubules. Thus even a small portion of the yolk-sac wall from any region reveals a number of masses of endoderm

cells forming spherical ovoid tubules. More villi and more tubules are added by the continued folding activity of the endoderm layer of the yolk-sac wall. Over the entire endoderm layer with its villi the mesoderm continues to form a covering layer of scattered and fusiform cells, one or two deep. The process of the folding of the wall and the formation of the large number of tubules becomes complete.

At mid-term stage the walls of the yolk-sac approximate and the yolk-sac lumen is very much reduced. The endoderm cells continue to form more villi. An examination of a small portion of the wall in any region shows a large number of such tubules each with a lumen (Pl. VII, Fig. 7). Two more points of interest may be mentioned. A vitelline capillary may be enclosed by the endoderm cells in a tubule (Pl. VII, Fig. 8). The tubule is thus lined by the endothelial cells of the capillary and endoderm cells. Such tubules contain blood corpuscles.

Towards the close of the gestation period, the cells of the tubules become very much large in size. The lumen of the tubule is beginning to be lost and thus cells which form the wall of a tubule become now scattered masses of cells; each mass is of three to four cells with very large nuclei. The nuclei of the cells are vacuolated (Pl. VIII, Fig. 9). It seems that the tubules break down into *syncytial masses*, each mass consisting of three to four cells whose outlines cannot be made out and having prominent large vacuolated nuclei. Such cell masses are separated from each other by intervening spaces.

A fully formed yolk-sac gland at full term (Pl. VII, Fig. 2, and Pl. VIII, Fig. 9) shows groups of syncytial cell masses whose glandular activity seems to have come to an end. Such syncytial masses contain within them mesodermal cells and an entire group of such a syncytial mass is covered over with mesodermal cells. In some of these groups a large vitelline capillary can be seen generally with or without blood corpuscles.

(b) *Cynopterus sphinx gangeticus* (Anderson):

General history of the yolk-sac.—In this bat the development and subsequent changes of the yolk-sac and its final disposition are similar to those in *Pteropus*. But the gland is very much smaller in consequence of the smaller size of the animal and the foetus. The yolk-sac assumes the form of a shrivelled bag and later lies below the placenta on the mesometrial side and finally becomes glandular (Pl. VIII, Fig. 12).

Histological changes in the yolk-sac wall.—The histological changes in the yolk-sac wall are similar to those in *Pteropus*.

The endoderm layer of the yolk-sac wall at the pre-limb-bud stage consists of slightly columnar cells with nuclei on the side of the yolk-sac lumen and vacuoles on the side on which mesoderm cells lie. The wall folds to form villi and some of these villi are formed round the vitelline capillaries (Pl. VIII, Fig. 10). The mesoderm cells retain their original position.

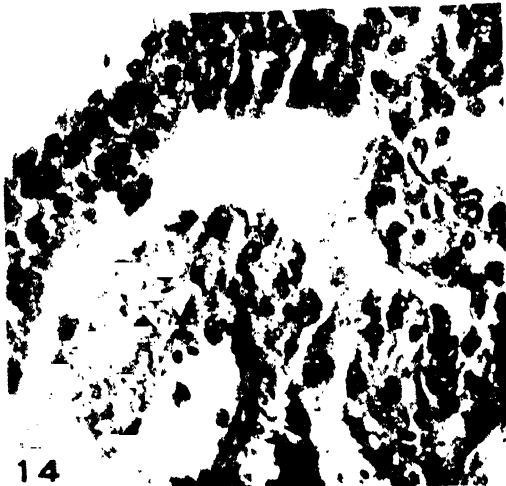
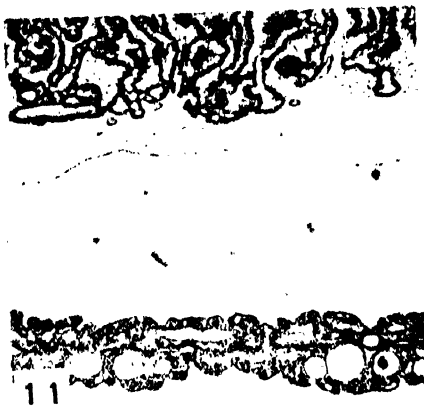
The lumen of the yolk-sac when it is finally transformed into a glandular structure still persists as slit-like discontinuous spaces till a late period of gestation (Pl. VIII, Fig. 11). On account of these slits the endoderm cells form a layer on each side of a slit. The endoderm wall folds to form villi. In its final condition the gland shows the same structure as in *Pteropus* (Pl. VIII, Fig. 12).

While there is thus a similarity in the changes leading to the transformation of the yolk-sac into a gland, it is formed very much earlier in gestation than in *Pteropus*.

(c) *Taphozous longimanus* (Hardwicke):

General history of the yolk-sac.—This is a microchiropteran species in which the yolk-sac becomes glandular as in Megachiroptera. The general history of the yolk-sac is similar to that of *Pteropus* and *Cynopterus*. In the early limb-bud





stage the yolk-sac becomes a shrivelled bag. The lumen is reduced and the bag lies below the placenta which in this animal is lateral. The endoderm layer folds to form villi. Some of these are formed round the vitelline capillaries. At a later stage, i.e. at mid-term stage, the tubules of the gland consisting of endoderm cells and surrounded by mesoderm cells lie close to each other. The lumen of the yolk-sac is reduced but not completely lost, and in a fully formed gland a bunch of tubules is separated from the adjacent bunch by spaces which are the remnants of the yolk-sac cavity.

Histological changes.—In the early limb-bud stage the yolk-sac is an elongated ovoid structure with folded walls (Pl. VIII, Fig. 13). The endoderm cells are columnar with spherical nuclei. Cells lie close together. Endodermal folds appear in some regions only. Some of these villi form closed tubules with lumina (Pl. VIII, Fig. 14). Fewer tubules are formed compared with those of *Pteropus* and *Cynopterus*. The mesoderm cells lie outside the endoderm cells and are fusiform in shape. They form a continuous covering to the endoderm layer.

In the late limb-bud stage endoderm villi are more numerous and some portions of the wall do not form any folds at all. The endoderm layer is thick, cells being four to five deep (Pl. VIII, Fig. 14) and since these and the villi project into the lumen of the yolk-sac wall, the lumen is further reduced. In a fully formed gland (Pl. IX, Fig. 15) the lumen of the yolk-sac persists and masses of the gland tubules are separated from each other by spaces which are the remnants of the lumen of yolk-sac. Each tubule consists of endodermal columnar cells surrounded by mesoderm cells. Some tubules enclose blood vessels lined by endothelium and contain nucleated blood corpuscles. In a fully formed gland some of the spaces are full of secretion.

(d) *Rhinopoma kinneari* (Wroughton):

General history of the yolk-sac.—In this microchiropteran bat belonging to the family Rhinopomidae the placentation is mesometrial which is unusual amongst the Microchiroptera. In this respect it resembles Megachiroptera. The yolk-sac wall has the same general history as in the other three species described. It shrivels, the walls are thrown into folds, and the lumen is considerably reduced. It later lies below the placenta. But as in *Taphozous* the original lumen of the yolk-sac is never completely obliterated.

Histological changes.—The yolk-sac is in the form of a shrivelled, ovoid, elongated bag, and the wall nearer the placenta folds to form villi (Pl. IX, Fig. 16) as in *Pteropus*, *Cynopterus* and *Taphozous*. But the other wall remains mostly without much change and whatever portion of it does fold, the villi formed are simple. The endoderm forms villi (Pl. IX, Fig. 17) which are then cut off from the endoderm wall which continues to form more villi. Thus a certain number of tubules are formed. The method of folding is the same as in the three foregoing species and, as in them, some of the tubules enclose blood capillaries with endothelial lining round which mesoderm is present. Some of the spaces between the tubules are filled with secretion as in *Taphozous*.

The endoderm is columnar and vacuolated forming a continuous layer. The mesoderm cells are fusiform. The endoderm cells are two to three deep. It will be noticed that the mesoderm cells in the region of the wall where villi are not formed form only a single layer of scattered fusiform cells which are more numerous round the villi and tubules. Finally, the yolk-sac is converted into a gland (Pl. IX, Fig. 18).

DISCUSSION

In all the foregoing species in which the yolk-sac wall has been studied the changes in the yolk-sac wall follow the same general plan. At a certain stage of development the splanchnopleurae of the yolk-sac is withdrawn from the area

occupied by the yolk-sac and the latter assumes the form of a shrivelled bag lying at first laterally and later below the chorio-allantoic placenta. In *Pteropus* and *Cynopterus* the placenta is mesometrial and the yolk-sac in the form of a shrivelled bag lies below the placenta on the mesometrial side. In *Taphozous* the placenta is lateral and the yolk-sac lies on the lateral side. In *Rhinopoma* the placenta is on the mesometrial side and the yolk-sac lies on that side.

In all cases the wall of the bag undergoes the same histological changes. In the first instance the endoderm folds to form villi. These become closed tubules, some of them enclosing blood capillaries with nucleated blood corpuscles (not enucleated as stated by Srivastava, 1952). More villi are formed. The endoderm cells are columnar with prominent nuclei. The mesoderm cells retain the same position. These are fusiform cells and not forming a continuous layer. In *Pteropus* the lumen is completely obliterated, in *Cynopterus* it persists only in the form of very small irregular slits, but in the two Microchiroptera the original lumen persists and runs between the bunches of the tubules of the gland. In *Rhinopoma*, however, the folding is much simpler, considerable portions of one wall not folding at all.

Towards the end of gestation the endoderm cells of the tubules of the gland hypertrophy and form masses. It seems that the secretory function of the gland comes to an end.

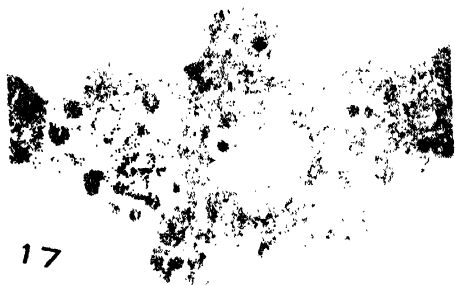
There are two aspects of the phenomenon connected with the conversion of the yolk-sac into a gland: (1) the physiology of the gland, and (2) its occurrence in both Mega- and Microchiroptera.

I propose to describe in a later paper the cyto- and histo-chemistry of the gland and the correlation between the development of the corpus luteum and the gland. I hope these studies may throw light on the probable function of the gland.

Here I shall confine my remarks to interordinal relationship of mammals. Mossman (1937, page 191) has suggested that the ancestral stock of placental mammals was characterized by the possession of 'antimesometrial disc, large yolk-sac and allantoic vesicle. Predominant chorio-allantoic placentation of broad zonary labyrinthine endothelio-chorial type'. From this ancestral stock the Mega- and Microchiroptera diverged along different lines: Megachiroptera characterized by the possession of 'free, solid gland-like y.s. All. v. (?) Mesometrial, laby. hemochorial placenta'. The Microchiroptera, on the other hand, representing another line in evolution and characterized by 'small all. v. Some have endothelio chor. aces. placentae'. The intermediate stage in this evolution of Microchiroptera possessed 'antimesometrial disc. Incom-inversion y.s. Antimeso. laby. hemochorial placenta'. Evidently, Mossman (1937) based this phylogeny on the basis of the facts known to him. In Microchiroptera, there are at least two species, *Taphozous longimanus* and *Rhinopoma kinneari*, belonging to two different families in which the yolk-sac becomes gland-like as in Megachiroptera and in one of them, viz. *Rh. kinneari*, even the site of placentation is mesometrial as in Megachiroptera and in the other, viz. *T. longimanus*, the placentation is lateral. It is possible that there may still be some other Microchiroptera whose embryology is not at present known, which may show characteristics in their development similar to those shown by *Taphozous* and *Rhinopoma* but different from those so far recorded in other Microchiroptera. I suggest that the Mega- and Microchiroptera are more nearly related to each other and such similarity as is shown by them is not a case of convergence in evolution.

ABSTRACT

The phenomenon of the conversion of the yolk-sac wall into a gland has been reported to occur in Megachiroptera but not in Microchiroptera. It has now been found to occur in two species of the latter group, viz. in *Taphozous longimanus* (Hardwicke) and in *Rhinopoma kinneari* (Wroughton). The process of conversion is essentially the same both in Mega- and Microchiroptera. The endoderm layer of the yolk-sac wall folds to form villi which become closed tubules. The endoderm cells of these tubules later become syncytial and, at term, their nuclei



become very large and vacuolar. Since a yolk-sac gland is formed in both groups of Chiroptera, they seem to be more intimately related to each other and the theory that their resemblance to each other is probably due to convergence in evolution needs revision.

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EXPLANATION OF PLATES VII-IX

All figures are photomicrographs.

Figs. 1 to 9. *Pteropus giganteus giganteus*.

- FIG. 1. Mid-term stage. The walls of the yolk-sac approximate, and hence the lumen of the yolk-sac is very much reduced. $\times 20$.
- „ 2. Sections of a fully formed gland removed from below the placenta prior to parturition. $\times 260$.
- „ 3. A portion of the vascular yolk-sac wall at the abembryonic pole on the antimesometrial side showing vacuolated columnar endoderm cells and fusiform mesoderm cells. $\times 350$.
- „ 4. A portion of the yolk-sac wall on the lateral side. $\times 350$.
- „ 5. A portion of the yolk-sac wall at late limb-bud stage to show the folding of the endoderm to form villi. $\times 350$.
- „ 6. Another portion of the yolk-sac wall. The folding is more pronounced. $\times 350$.
- „ 7. Yolk-sac wall showing endoderm tubules. $\times 350$.
- „ 8. A tubule which has surrounded a capillary. $\times 350$.
- „ 9. A portion of the fully formed gland showing break-down of tubules, the endoderm cells forming syncytial masses of three to four cells; the mesoderm cells are on the outside of one such portion and also occur between the masses. $\times 350$.

FIGS. 10 TO 12. *Cynopterus sphinx gangeticus*.

- FIG. 10. The folding of the endoderm to form villi. Mesoderm cells are outside the tubules. $\times 350$.
,, 11. Yolk-sac gland at late stage lying below the placenta. Note slits representing the lumen of the yolk-sac. $\times 40$.
,, 12. Fully formed yolk-sac gland. $\times 350$.

FIGS. 13 TO 15. *Taphozous longimanus*.

- FIG. 13. Early limb-bud stage to show the two walls of the yolk-sac approximating each other and thrown into folds. $\times 40$.
,, 14. Endoderm tubules are formed. $\times 350$.
,, 15. Fully formed gland. $\times 350$.

FIGS. 16 TO 18. *Rhinopoma kinneari*.

- FIG. 16. Yolk-sac in the form of a shrivelled bag. A considerable part is free from folding. $\times 20$.
,, 17. Endoderm folding to form villi. $\times 350$.
,, 18. Fully formed gland. $\times 130$.

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SOME OBSERVATIONS ON THE FOETAL MEMBRANES OF
THE INDIAN PALMCIVET, *PARADOXURUS HERMA-
PHRODITUS HERMAPHRODITUS*
(SCHRATER)

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(Received February 15 ; approved for reading on May 4, 1956)

INTRODUCTION

A perusal of literature on the foetal membranes of the Carnivora reveals that, while extensive work has been done on the common carnivores, little is known of the early development and placentation of wild forms particularly of some families. A study of the development of carnivores is all the more interesting, because even amongst the forms whose embryology has been studied, there are differences in the morphology of the foetal membranes. Further, due to paucity of information on a number of families, attempts to explain phylogeny and inter-relationships of the carnivores can only be regarded as tentative and even unsatisfactory. This is obviously due to the difficulty of obtaining material of wild forms for study. Viverridae is one of such families of the Carnivora and includes two subfamilies—Viverrinae and Paradoxurinae. The only record of the description of foetal membranes of Viverridae is by Strahl (1905) on the foetal membranes of *Viverra civetta*. The present study on the foetal membranes of a species of *Paradoxurus* belonging to the subfamily Paradoxurinae may prove to be of interest and value as it is the first account of the foetal membranes of a member of this subfamily.

Since the publication of observations on the placenta of dog by Bojanus (1820), several workers have studied the development and placentation of some fissipede carnivores. Animals which have been studied are dog (Duval, 1893 and 1894 ; Bonnet, 1897, 1901 and 1902 ; Strahl, 1890), cat (Duval, 1894 and 1895 ; Strahl, 1905 ; Mossman, 1937 ; Hill and Tribe, 1924), racoon (Watson, 1881), ferret (Bischoff, 1845 ; Strahl, 1889 ; Strahl and Ballmann, 1915), zorilla (Rau, 1925), *Viverra civetta* (Strahl, 1905), brown bear (Rau, 1925), tiger (Srivastava, 1952), and *Felis (-Panthera) leo* (Mossman, 1937). The facts recorded by the foregoing authors may be briefly summarized as follows :—

Canidae : Ripe placenta is in the form of broad belt (zonary) occupying the equatorial region of the gestation sac. There is a haematoma band on each of the two margins of the placental belt. The placenta is lobular, labyrinthine and endotheliochorial. The allantoic vesicle is large. The yolk-sac placenta is well developed during early stages of development ; later, the yolk-sac splanchnopleure is withdrawn from the placental site, and the yolk-sac becomes a collapsed bag.

Procyonidae : The condition of the yolk-sac is similar to that of the dog. The placenta is zonary and endotheliochorial, but the placental belt is incomplete on the dorsal aspect of the embryo. Haematoma on the margins of the placental belt are absent.

Mustelidae : In early morphogenesis, the chorio-allantoic placenta of ferrets is zonary, but in late stages it assumes the form of two mesometrial discs. There is but a single haematoma on the antimesometrial side. The placenta is labyrinthine and endotheliochorial. In *Zorilla striata*, however, Rau (1925) describes the presence of two haematoma, one lateral to each placental disc, the median haematoma being absent.

Ursidae : Placenta is zonary, labyrinthine and endotheliochorial without marginal haematoma.

Felidae : Placenta is zonary, as in dog, lamelliform and endotheliochorial. Marginal haematoma are present but very imperfectly developed.

Viverridae (subfamily *Viverrinae*) : Placenta is annular, zonary, labyrinthine and endotheliochorial. Nothing is known of the condition of haematoma.

The facts summarized above indicate that in the *Carnivora* the placenta is generally zonary in shape, labyrinthine and endotheliochorial. Only in *Mustelidae* and *Ursidae*, it is not in the form of a belt, but it is disc-shaped, double in *Mustelidae* and single in *Ursidae*. In *Procyonidae*, the placental girdle is incomplete on the dorsal side of the embryo. These are also variations in the disposition and in the extent of the development of haematoma. In *Canidae*, they are well developed, while they are poorly developed in *Felidae*. In *Mustelidae*, the haematoma occur either on the lateral side of each disc or as an antimesometrial patch. In *Ursidae*, the haematoma form a ring round the margin of the single placental disc and in *Procyonidae*, the marginal haematoma are absent.

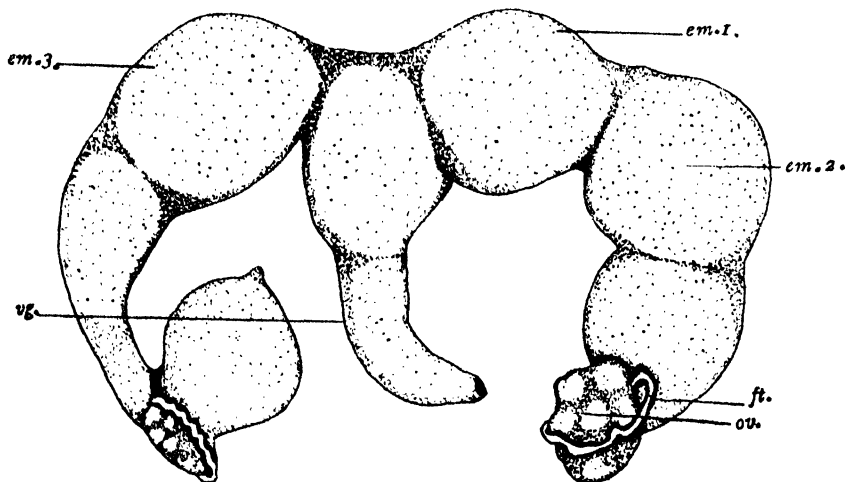
MATERIAL AND METHODS

A pregnant specimen of *Paradoxurus hermaphroditus hermaphroditus* was captured in Nagpur on 16th May, 1954. There were three embryos, one in the right horn and two in the left horn of the uterus. The uterus with embryos *in situ* was fixed in alcoholic Bouin's fluid after injecting some fixative through the cervix into the uterus. The material was transferred to fresh fixative every day for four days and later it was preserved in 70% alcohol.

The usual method of dehydration by passing the specimens through graded alcohols and later clearing and embedding in paraffin was employed. Both vertical and transverse sections of separate gestation sacs were cut. Sections were prepared with Ehrlich's haematoxylin counterstained with eosin.

OBSERVATIONS

(a) *Reproductive organs*.—The uterus (Text-fig. 1) is bicornuate as in all other carnivores. The left horn contained two embryos and the right horn one. All the

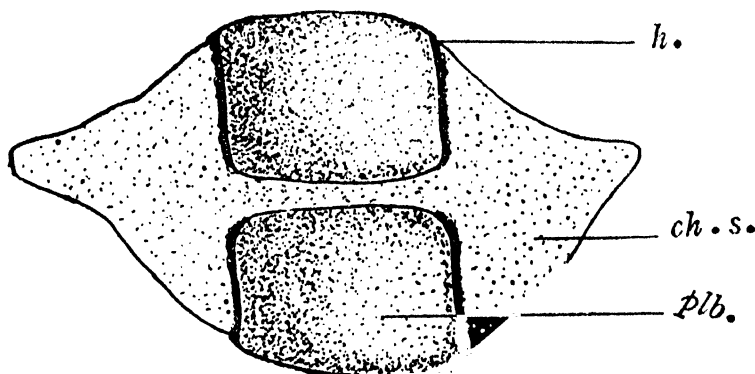


TEXT-FIG. 1. Female reproductive organs of *Paradoxurus h. hermaphroditus* (ventral view).
× ca. 3.

em. 1, 2, 3, embryos; ft., fallopian tube; ov., ovary; vg., vagina.

three uterine swellings were nearly of the same size being approximately 3.2 cm. in diameter. The head ends of embryos were directed towards the cervix, and the dorsal side of each was against the lateral side of the uterus. All the embryos were practically at the same stage of development, and each measured 2.6 cm. in length from the tip of the snout to the base of the tail. The embryos were fairly advanced in development showing the limbs, toes and ear pinna. A few vibrissae were seen on the snout. Sections revealed that erythrocytes were enucleate and the long bones had commenced to ossify.

(b) *Foetal membranes*.—The general appearance of the chorionic sac after it was peeled from the uterine wall is shown in Text-fig. 2. The placenta is in the



TEXT-FIG. 2. Chorionic sac of *Paradoxurus h. hermaphroditus* to show the placental band, the position of haematoma and the placental gap. \times ca. 30.
ch. s., chorionic sac; h., haematoma; plb., placental band.

form of a uniform wide belt or girdle measuring 2.0 cm. in width, round the equatorial part of the chorionic sac. The placental girdle is not complete, there being a gap on the antimesometrial side (Pl. X, Fig. 1, *plg.*) measuring 0.5 cm. in width. The two margins of the placental belt are darker in colour, and these indicate the position of haematoma which, in this carnivore, are poorly developed.

The general disposition of the foetal membranes is shown in Pl. X, Fig. 1, which represents a transverse section of one of the three gestation sacs. The foetus lies in the centre of the uterine cavity slightly towards the antimesometrial aspect. The amnion is closely adherent to the body of the foetus, and on the antimesometrial side, it is also adherent to the inner wall of the allantoic vesicle (*j.*), resulting in the formation of a common membrane. The yolk-sac (*ys.*) is disposed on the mesometrial side as a collapsed bag with wrinkled walls. The allantoic vesicle (*all. v.*) is large and the allantochorion is apposed to the placental surface. The placenta has the shape of a horse-shoe and it receives blood capillaries on the entire surface through the allantochorion. The placenta is almost uniform in thickness. The space (Pl. X, Fig. 1, *x.*) outside the placental zone between the placenta and the uterine muscularis is an artifact caused by shrinkage during fixation and dehydration.

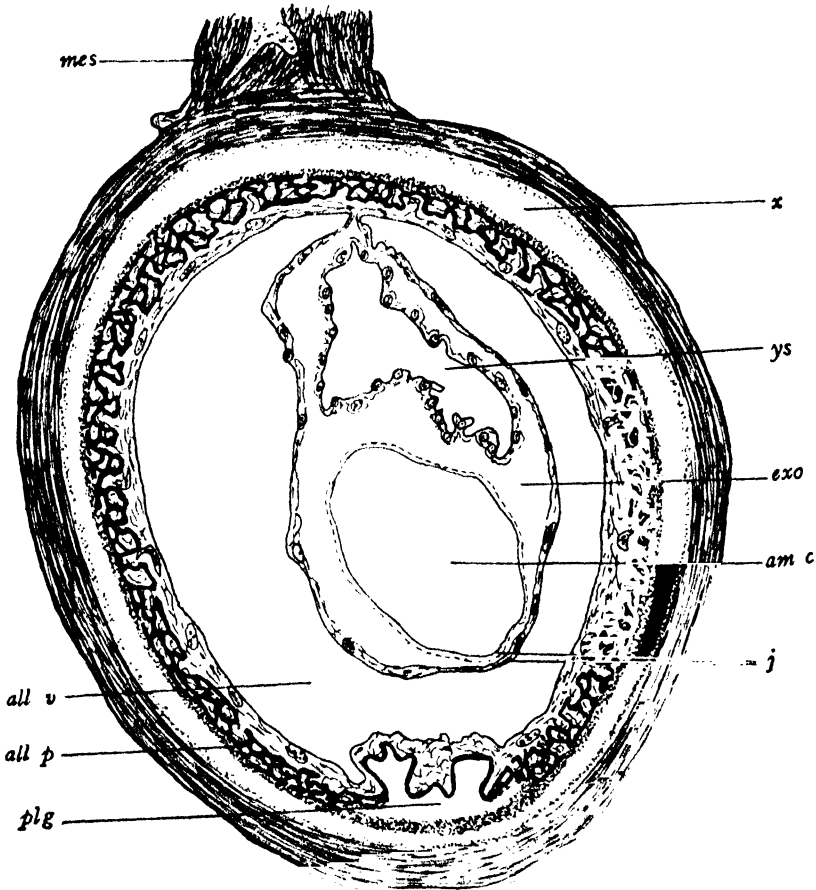
Amnion : The amnion is a thin bilaminar membrane enclosing the foetus. It is attached to the base of the umbilical-cord and is free on all sides except on the antimesometrial where it is closely adherent to the inner wall of the allantoic vesicle (*j.*). The amnion membrane consists of an inner ectodermal layer of highly flattened cells appearing fusiform in sections, with fusiform nuclei and containing granular cytoplasm and an outer mesodermal layer with loosely arranged flattened cells. Only the part of the amnion layer which is adherent to the inner wall of the allantoic vesicle is vascular owing to the presence of allantoic capillaries.

Yolk-sac : This lies as a shrivelled bag on the mesometrial aspect of the gestation sac. The yolk-sac splanchnopleure (Pl. X, Fig. 2) is profusely supplied with vitelline

vessels (*vit. v.*) whose presence gives the yolk-sac wall a beaded appearance. The mesodermal layer of the yolk-sac is often 3-4 cells deep and is made up of fusiform flattened cells with fusiform nuclei. The endodermal lining is uniformly a one-celled membrane with cubical cells and spherical nuclei.

Allantois : The wide allantoic vesicle (*all. v.*) completely obliterates the exocoelom except in the regions where the inner wall or the foetal surface of the allantoic vesicle is not in contact or cohesion with either the amnion or the yolk-sac. The (foetal) wall of the allantoic vesicle (Pl. X, Fig. 3) is made up of an inner endodermal layer of flattened to semi-cubical cells reinforced by allantoic mesoderm (*m.*) consisting of loosely arranged flattened fusiform cells. Blood capillaries occur in several places in this the inner wall of the allantoic vesicle. Very often the endodermal cells of the allantois can be recognized only by topography and not by any definite structural characteristics, since they almost resemble the mesodermal cells of the allantoic. Its cohesion with the amnion has already been referred to.

On the other hand, the placental wall of the allantoic vesicle (Pl. X, Fig. 4) shows a clear distinction between endodermal (*en.*) and mesodermal (*m.*) cells comprising it. The former not only form a uniform layer, but are distinctly semi-cubical, whereas the mesoderm shows piled up clusters of cells which are invariably flattened



TEXT-FIG. 3. Transverse section through one chorionic sac of *Paradoxurus h. hermaphroditus* to show the general disposition of foetal membranes. \times ca. 70.

am. c., amniotic cavity; *all. p.*, allantoic placenta; *all. v.*, allantoic vesicle; *exo.*, exocoelom; *j.*, wall of amniotic cavity adhering to the wall of the allantoic vesicle; *mes.*, mesometrium; *plg.*, placental gap; *x.*, space which is artifact; *ys.*, yolk-sac.

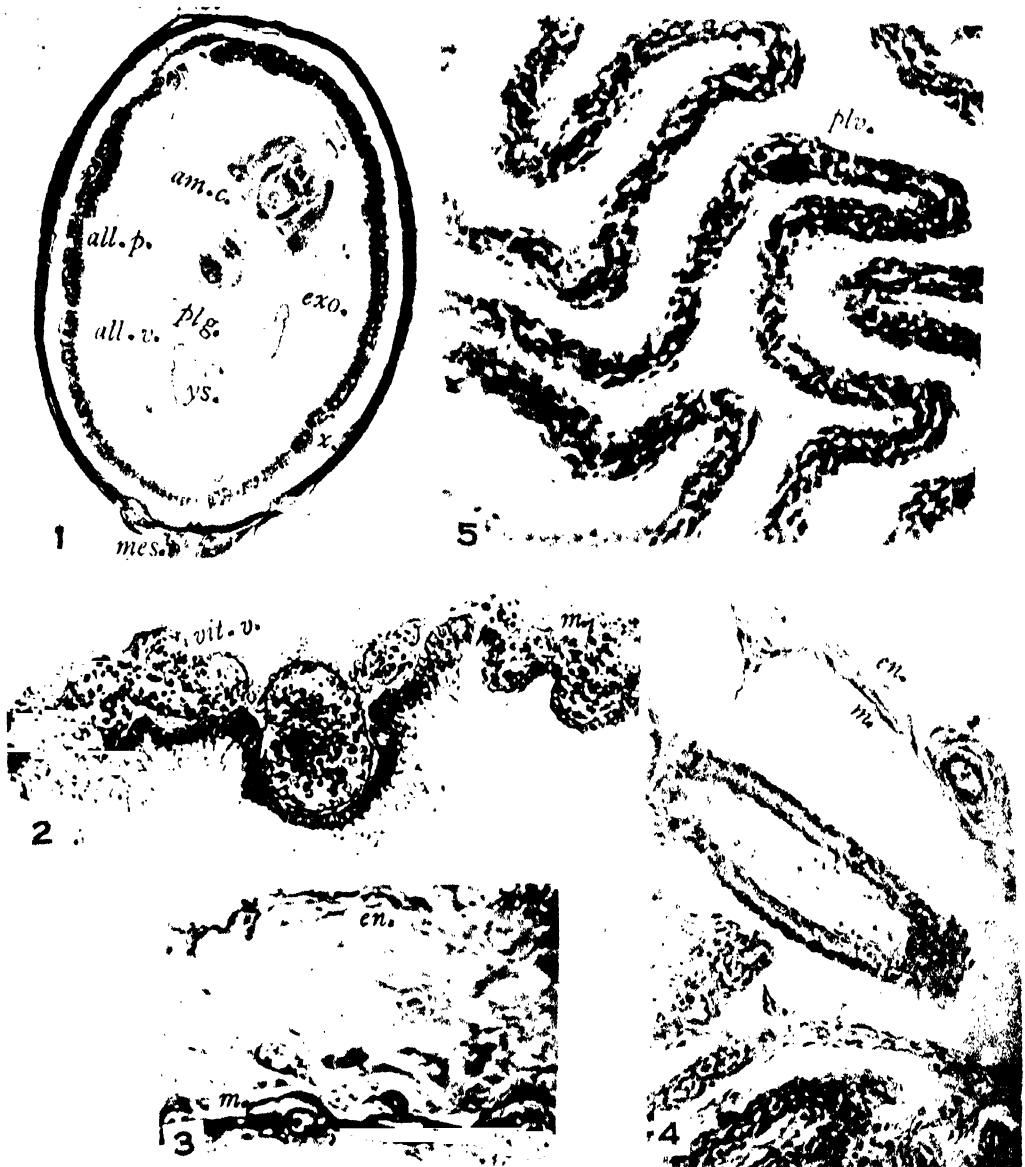


FIG. 1. T.S. of the gravid uterus through the placental band. $\times 2$.
 " 2. Yolk-sac splanchnopleure. $\times 70$.
 " 3. Foetal wall of the allantoic vesicle. $\times 145$.
 " 4. Placental wall of the allantoic vesicle. $\times 145$.
 " 5. Placental villi. $\times 70$.

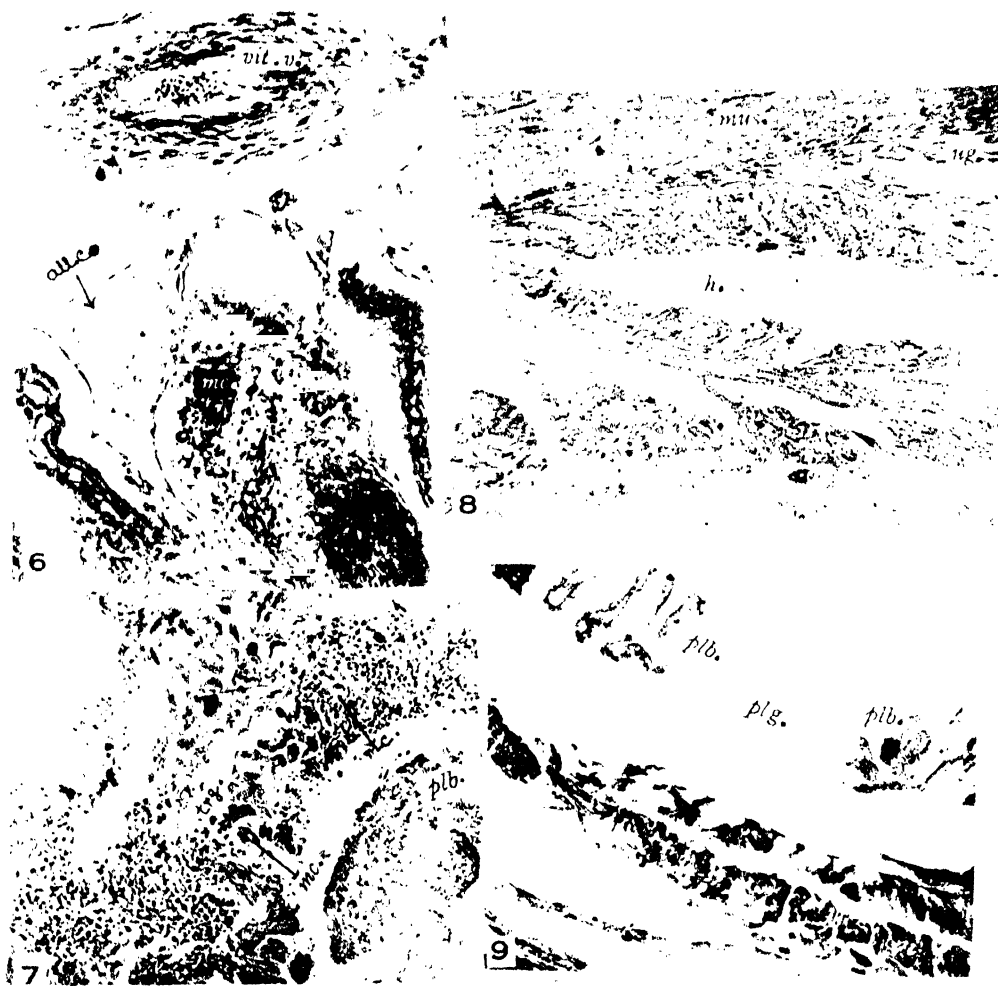


FIG. 6. A villus of the placenta showing enclosed maternal capillary. $\times 145$.
 .. 7. The region outside the placental zone. $\times 70$.
 .. 8. Margin of the placenta where there should be haematomal band. $\times 145$.
 .. 9. Placental gap. $\times 25$.

or stellate. This mesodermal layer with its loosely arranged cells and carrying allantoic capillaries enters into spaces of the placental labyrinth (Pl. X, Figs. 4 and 5).

Placenta : The placenta is formed on all sides of the uterus as a belt excepting on the strictly antimesometrial side where there is a small gap (Text-fig. 2).

In a transverse section of the uterus passing through the placenta the following regions can be made out: (1) placental labyrinth, (2) transition zone, (3) deep glandular zone, and (4) muscularis. The placenta is horse-shoe-shaped.

The foetal surface of the placenta is lined by the allanto chorion which shows the presence of a large number of allantoic capillaries (Pl. XI, Fig. 6). The allanto chorion (*all. c.*) with its capillaries enters the placental labyrinth. Thus, the placental labyrinth presents a very complicated appearance as it consists of branching and interlacing villi disposed radially (Pl. X, Fig. 5). The villi are in the nature of flakes or lamellae hanging from the uterine wall, and only at their distal extremity (i.e. towards the foetal surface of the placenta) are the lamellae unbranched. The lamella or the villus in section is made up of syncytical trophoblast. The core of each villus contains a maternal capillary (Pl. XI, Fig. 6, *mc.*) which is lined by endothelial cells, and immediately surrounding it, there is a trophoblast layer containing scattered nuclei. At the periphery of the villus, however, the nuclei are large and are arranged in rows. Thus, the trophoblast surrounding the maternal capillaries inside the villi can be distinguished into two regions: an inner hyaline layer with scattered larger nuclei surrounding the endothelium of the capillary and an outer granular region with spherical nuclei arranged in a regular row.

The endothelial cells lining the maternal capillaries inside the villi are of the usual type: cuboidal with spherical large nuclei. The maternal capillaries are larger on the foetal side of the placenta but become smaller towards the transition zone, (Pl. XI, Fig. 7, *mc.*) and the endothelial cells also become more flattened. No giant cells were observed in any part of the placenta.

Outside the zone of the placental labyrinth the tissue is rather weak and usually tears off during fixation and dehydration. In this transition zone (Pl. XI, Fig. 7) the ends of the placental trophoblastic lamellae unite forming a sheet of syncytiotrophoblast, and the endometrial tissue surrounding it consists of degenerating cells with indistinct boundaries. The nuclei, however, are prominent and irregular in shape. Maternal capillaries are present in this region.

Outside of this transition zone, a region of the endometrium lines the muscularis. Highly hypertrophied remnants of uterine glands (Pl. XI, Fig. 8, *ug.*) with large lumina occur in this region. Gland cells are large and cuboidal with vesicular nuclei. The muscularis consists mostly of circular muscles enclosing wide spaces.

The haematoma are very poorly developed in *Paradoxurus* and are covered with trophoblast and enclose a small quantity of extravasated blood (Pl. XI, Fig. 8). The condition resembles that of a cat. The placental gap is quite well defined (Pl. XI, Fig. 9, *plg.*).

DISCUSSION

Though a detailed discussion on the development and placentation of *P. h. hermaphroditus* is not possible from observations recorded in this paper, based as they are on one stage of advanced development, it would be of interest to mention a few obvious peculiarities. The general disposition of the foetal membranes is typically carnivorous—shrivelled yolk-sac, large allantoic vesicle and its inner wall adherent to the amnion at some places. The allantoic vesicle deviates a little from the condition found in other carnivores. The placental belt is incomplete on the antimesometrial side, so that in transverse sections, placenta has the shape of a narrow horse-shoe resembling that of *Procyon lotor* (Watson, 1881). The poor development of marginal haematoma in the palmcivet also resembles the condition in *P. lotor*. It is likely that during the early stages of development, the placental band is complete and becomes incomplete as pregnancy advances.

While discussing the evolution of the placenta in the Carnivora, Rau (1925) has stated that, on palaeontological evidence, Mustelidae, Ursidae, Procyonidae and Canidae form one line of evolution and Viverridae, Hyaenidae and Felidae represent another line. In Canidae and Felidae, the placenta is completely zonary, but haematoma are well developed in the former and poorly developed in the latter. There is a distinct tendency for the placenta to become lobular in Canidae, but not so in Felidae. Thus, there is considerable justification for regarding Felidae and Canidae as forming different lines of evolution. In other families so far studied there are interesting variations in the morphology of the placental band, incomplete in Procyonidae, reduced to a double discoid condition in Mustelidae, and a single disc in Ursidae. However, in all these families the original condition of the placenta, when first formed, is zonary and the zonary condition is reduced, as development proceeds, to a double discoid or a single discoid condition. Rau (1925) also maintains that the placenta of Mustelidae is structurally more efficient as an organ of foetal nutrition than that of a dog or a cat, and the placenta of bear is more efficient than that of Mustelidae.

The placental morphology of *Paradoxurus* resembles closely that of Procyonidae. The occurrence of lamellar placental labyrinth connects Viverridae with Felidae. Nothing is known of the placentation in Hyaenidae,* and hence it is not possible to determine the relationship of Viverridae with Hyaenidae. At the most, it may be suggested that specialization of placenta in the canid line of evolution also occurs in the felid line on the assumption that Viverridae is included in the latter as is warranted by studies on comparative anatomy and palaeontology. But the haematoma are absent in both Procyonidae and Paradoxurinae. Nothing is known of the condition of haematoma in Viverrinae, *Viverra civetta*. If on further examination it is found that the haematoma are absent in Viverrinae as in Paradoxurinae (both belonging to family Viverridae), then Procyonidae is more allied to Viverridae and Felidae and should be included in this line of evolution. But at present, there are wide gaps in our knowledge of the placenta of Carnivora, and hence any attempt to suggest lines of evolution and relationship (Rau, 1925) should be regarded as tentative.

ACKNOWLEDGEMENT

I am grateful to my colleague, Mr. A. Gopalakrishna, for assistance in the preparation of this paper.

SUMMARY

The foetal membranes of the subfamily Paradoxurinae of the family Viverridae are not known, and this paper contains a few observations on a member of this subfamily *Paradoxurus hermaphroditus hermaphroditus*. The amnion is a thin non-vascular membrane except where it is in contact with the vascular inner wall of the allantoic vesicle. The yolk-sac is a collapsed bag hanging on the mesometrial side. The allantoic vesicle is large and practically obliterates the exocoelom. The allantoic placenta, though zonary, shows a marked gap on the anti-mesometrial side. The placenta is endotheliochorial and lamelliform with the lamellae arranged radially. The haematoma are absent.

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* Srivastava gave an account of the placenta in *Hyaena striata* at the Zoology Section of the Indian Science Congress Association meeting in 1953. His results have not been published. In the abstract of the paper it is stated that the haematoma are absent.

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ABBREVIATIONS USED IN PLATE FIGURES

am. c., amniotic cavity ; *all. c.*, allantochorion ; *all. p.*, allantoic placenta ; *all. v.*, allantoic vesicle ; *en.*, endoderm ; *exo.*, exocoelom ; *h.*, haematoma ; *j.*, place where the wall of amnion adheres to the wall of the allantoic vesicle ; *m.*, mesoderm ; *mc.*, maternal capillary ; *mes.*, mesometrium ; *mus.*, muscularis ; *plb.*, placental band ; *plg.*, placental gap ; *pl. v.*, placental villi ; *sy.*, syncytiotrophoblast ; *trz.*, transitional zone ; *ug.*, uterine gland ; *vit. v.*, vitelline vessel ; *ys.*, yolk-sac ; *x.*, space which is artifact.

Issued November 15, 1956.

ON THE DEVELOPMENT AND PLACENTATION OF A
MEGACHIROPTERAN BAT—*CYNOPTERUS SPHINX*
GANGETICUS (ANDERSON)

by M. A. MOGHE, F.N.I., Department of Zoology, Poona University, Poona 7

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INTRODUCTION

In a previous paper (Moghe, 1951) I described the early stages of development and the structure of the placenta of a species of Pteropodidae, *Pteropus giganteus giganteus* (Brünnich). A brief account of the previous work on the embryology of Megachiroptera was also included in the paper. In this paper, I propose to describe some stages of development of another species of Pteropodidae, viz. *Cynopterus sphinx gangeticus* (Anderson). While in many respects the embryology of this species agrees with that of other Megachiroptera, it, nevertheless, presents some peculiarities which seem to be interesting. Keibel (1922) described the development of *Cynopterus marginatus* and stated that, though *Xantharpya amplexicaudata* described by Kohlbrugge (1913) and *Pteropus edulis* described by Selenka (1892) and by Göhre (1892) are allied to *Cynopterus* and belong to the same sub-family, his observations on *Cynopterus* were different from those of Kohlbrugge, Selenka and Göhre. Keibel described the early stage of development, formation of primitive amniotic cavity, and the part played by trophoblast in the initial stages of the formation of the placenta. The implantation and amniogenesis of *C. marginatus* differ from other Megachiroptera.

The morphogenesis of foetal membranes in Chiroptera has been summarized by Mossman (1937, p. 207). Regarding amniogenesis, he states that in Megachiroptera it is formed by cavitation and the early features are alike in both species (viz. *P. edulis*, *C. marginatus*). In the description of figures in Plate 8 he further comments on the general inadequacy of descriptions relating to the development of Megachiroptera.

Iyer (1949) in a report submitted to the National Institute of Sciences of India on the reproduction of *Cynopterus sphinx sphinx* (Vahl) observes, 'This bat is probably polyoestrous and that the statement of Marshall (1922) that bats are not polyoestrous is not strictly correct.'

Phillips (1924) made the following observation on *Cynopterus brachyotus ceylonensis*, 'In Passera, females have been found with young in May and again at the end of August. Out of four shot on August 29th one had a young one at the breast, while the other three had not yet brought forth. They were living in company of a young male but the old males were living by themselves' (p. 14).

I do not know of any other work on the breeding seasons or the development of any of the species of *Cynopterus*. In view of the very inadequate data available, I think that the observations recorded in this paper will add to our knowledge of the subject.

MATERIAL AND METHODS

Specimens were collected in and around a radius of about 100 miles of Nagpur (Madhya Pradesh, India). The uteri of both sides with other parts of the female genitalia were fixed in alcoholic Bouin's fixative. Both horns of the uteri were sectionised except in cases of noticeable and advanced pregnancies. Fifty-two specimens were examined and it was possible to obtain conceptuses in nearly all

stages of development, except early stages of cleavage of the egg. Uteri with the contained embryos were also sectionised. Uteri containing embryos in very advanced condition of development were cut open so as to observe the arrangement of the foetal membranes.

The following table gives the data of collection and the stage of development :—

Date.	Number of females.	Condition of uterus.
10-1-1948	2	Foetus in the right horn of the uterus in one specimen and in the left horn in the other. In both the cases embryo beyond the late limb-bud stage.
13-1-1948	3	Pregnancy in right horn in all cases. Foetus beyond the late limb-bud stage.
15-1-1948	2	No pregnancy in one specimen. Pregnancy in the left horn in the other. Foetus beyond the late limb-bud stage.
10-2-1948	1	Non-pregnant.
11-2-1948	5	In all cases pregnancy in right horn. Foetuses in advanced stage of development.
No specimens could be collected during the months of March, April, May and June. Several female specimens were collected during July, but in a majority of these, young ones were attached to the breasts and the mammary glands were large and in full lactation. Specimens collected during August and September were non-pregnant. No specimen was collected in October.		
8-11-1948	1	Pregnancy in right horn. Medullary groove stage.
11-11-1948	1	Pregnancy in left horn; bilaminar blastocyst with primary amniotic cavity.
13-11-1948	2	Pregnancy in right horn in one specimen and in left horn in the other. Stage slightly younger than the previous stage.
16-11-1948	1	Pregnancy in left horn, prochordal plate stage.
27-11-1948	1	Pregnancy in left horn, embryo more advanced in development than the previous stage.
28-11-1948	5	Four pregnant, pregnancy in right horn in three cases and in left horn in one. Condition more advanced than in specimens collected on 11-11-1948.
5-12-1948	2	Foetus in one in the prochordal state and in the other in the medullary groove stage.

In addition to the specimens listed above in the table, I collected 6 specimens on 3-12-1947. All these showed more advanced stages of pregnancy than those collected on 28-11-1948.

It is evident from the above data that specimens collected in November show early pregnancy. Probably, fertilization takes place late in October or early in November. Advanced pregnancy is found in specimens collected in January. Parturition takes place late in February or in early March. The period of gestation is about 115 to 125 days.

Specimens collected on 5-12-1948 are practically at the same stage of pregnancy as those collected on 8-11-1948 and 16-11-1948. It is evident that all females do not become pregnant during the same period.

EARLY STAGES OF DEVELOPMENT

Blastocyst I (13-11-1948) :

The bilaminar blastocyst from one of the two specimens collected on this date (Pl. XII, Fig. 1) occupies 30 sections, each 10μ thick. The embryonic mass is nearly spherical in shape and contains within it a cavity filled with cell detritus. This is the primary amniotic cavity. The endoderm is well differentiated and forms an inner lining to the bilaminar blastocyst. The endoderm cells beneath the embryonic mass are spherical and only one-cell deep (Pl. XII, Fig. 3), in the extra-embryonic region they are fusiform in shape but also one-cell deep.

Surrounding the blastocyst is a thick shell which takes a deeper stain than the rest of the uterus. The shell is formed of syncytiotrophoblast (Pl. XII, Fig. 2) recognized by the presence of scattered nuclei without cell outlines, and into which the basal layer of trophoblast has penetrated in the form of strands which occur in the form of villi. In the syncytiotrophoblast are spaces in which maternal blood corpuscles are present. In some places these blood spaces are lined by hypertrophied maternal endothelium.

Outside the shell there is a cavity between it and the muscular layer of the uterus on the antimesometrial and the lateral sides. This cavity contains the remnants of the uterine glands which have disappeared or which have undergone hypertrophy. This cavity is most probably not the uterine lumen though owing to the complete disappearance of the uterine glands and endometrium in some regions, a space devoid of any tissue is left which looks like the uterine lumen. On the mesometrial side the endometrium containing hypertrophied uterine glands is present between the placental shell and the myometrium of the uterus. If the space outside the placental shell on the antimesometrial side is not uterine lumen, it is evident that implantation is superficial and not intermediate.

An interesting feature of the blastocyst is the presence of a spongy tissue between the trophoblast and the embryonic mass and between the trophoblast and the endoderm. It consists of squamous vacuolated cells with nuclei appearing as black dots (Pl. XII, Figs. 1 and 3). These cells are connected with each other by protoplasmic strands. The tissue may be designated the endoderm-mesoderm tissue.

Blastocyst II (27-11-1948) :

The embryo is nearly at the same stage of development as in Blastocyst I. The following variations may be mentioned :

(i) The spongy tissue is more abundant. Spaces or clefts are noticeable in this tissue. One such cleft is seen in the photomicrograph (Pl. XII, Fig. 4).

(ii) Outgrowths from the basal trophoblast have now penetrated deeper so that the shell is thicker. The maternal blood spaces are lined by trophoblast, though some are still lined by hypertrophied endothelium. The region of the uterine wall outside the shell is very much reduced.

(iii) The primary amniotic cavity is very much larger, but is still filled with cell detritus.

(iv) The endoderm layer below the embryonic mass is more than one-cell deep.

Blastocyst III (11-11-1948) :

This is an extremely interesting stage from the point of view of amniogenesis. The cavity in the embryonic mass seen in blastocysts I and II filled with cell detritus

is the primitive amniotic cavity formed by cavitation. There is now another cavity formed (Pl. XII, Fig. 5) above the primary amniotic cavity on the mesometrial side occupying the space previously filled by the spongy tissue which, in this region, has either disappeared or moved laterally. The embryonic ectoderm in Blastocyst II is thin and about 2-cell deep. But in this stage the ectoderm is very much thicker. Folds from the edges of the embryonic ectoderm appear and close over the primitive amniotic cavity forming thereby a new cavity. The two cavities are not in communication with each other, but are separated by the cell layer forming the roof of the primitive amniotic cavity. The new cavity recalls to mind the epamniotic cavity of certain rodents, but this term is inapplicable to this new cavity. It may be called the secondary amniotic cavity. The mode of origin of the new cavity, if it has been correctly interpreted by me, denotes the formation of amniotic cavity by cavitation coupled with the formation of embryonic folds separating the primary amniotic cavity from a more mesometrially placed secondary cavity. Blood corpuscles are not found in any of the two cavities.

The yolk-sac wall is still bilaminar. The spongy tissue has now considerably extended laterally. The shell shows the same structure as in the preceding stage.

Blastocyst IV (18-11-1948) :

The secondary amniotic cavity and the primitive amniotic cavity are continuous (Pl. XII, Fig. 6) owing to the breakdown of the roof of the primitive amniotic cavity. The spongy tissue spreads laterally and forms a layer between the trophoblast and the endoderm excepting in a small antimesometrially situated abembryonic segment. The spongy tissue also lies between the trophoblast and the embryonic mass. But in addition to this tissue very distinct mesodermal cells are also seen. It is possible that the spongy tissue contributes to the formation of the mesoderm.

PROCHORDAL PLATE STAGE

I have two gravid uteri (16-11-1948 and 5-12-1948) in which the embryonic ectoderm is in the form of a plate (Pl. XII, Fig. 7). The plate is made up of cells, 4-5 deep. The plate shows the medullary plate thickening. The endoderm lies below it and between the two lies the mesoderm. The cells which are larger than the endodermal cells are irregular in shape and are joined to each other by cytoplasmic strands. The spongy tissue seems to have disappeared—only some remnants can be seen in addition to mesoderm cells in the wall of the yolk-sac.

The placental shell shows a greater abundance of cytotrophoblastic villi. Endothelium of maternal capillaries has become completely lost from all regions of the placenta. Outside the placental zone in the shell there is a thin layer of maternal endometrium. On the antimesometrial and lateral sides the placental shell as a whole is separated from the uterine wall by a space which is here and there traversed by strands of endometrial tissue.

MEDULLARY GROOVE STAGE

This stage was obtained from specimens collected on 27-11-1948 and 5-12-1948. The embryonic region occupies about 160 sections each 10μ thick. It consists of an ectodermal plate with medullary groove (Pl. XIII, Fig. 8). The mesoderm is still a solid layer of cells, 5-6 deep, in the embryonic region and 2-3 deep along the trilaminar wall of the yolk-sac. The mesoderm layer has reinforced the amnion also.

In the placental shell the cytotrophoblastic cords are profusely branched. The rest of the placenta consists of syncytiotrophoblast and maternal blood.

The rudiment of the notochord can be made out. The spongy tissue has completely disappeared.

The yolk-sac wall is completely trilaminar, mesoderm being present even in the small abembryonic portion of the yolk-sac wall on the antimesometrial side.

LATE LIMB-BUD STAGE (3-12-1947)

Between this stage and the preceding one there is a large gap. As in *Pteropus*, the entire yolk-sac splanchnopleure is withdrawn and lies loosely coiled on the lateral side below the allantoic placenta (Pl. XIII, Fig. 9). This is the stage which precedes the conversion of the yolk-sac into a gland.

The placenta appears horse-shoe-shaped in a transverse section. It is thicker in the middle and narrows at the two ends of the horse-shoe. The detailed structure of the placenta, except for its overall size, is the same as in *Pteropus*. There is a network of cytotrophoblastic tubules containing maternal blood corpuscles; and between the tubules lie the allantoic mesenchyme and foetal capillaries (Pl. XIII, Fig. 10).

NEAR-TERM STAGE (13-1-1948)

The important features of this stage are the conversion of the yolk-sac into a gland-like structure and the formation of the definitive placenta.

In the preceding stage (Pl. XIII, Fig. 9) the yolk-sac was loosely coiled. The lumen had not yet disappeared. The walls now come near each other, the lumen is nearly obliterated and persists as narrow spaces (Pl. XIII, Fig. 11). The yolk-sac gland lies below the allantoic placental disc. The structure of the yolk-sac gland is very similar to that of *Pteropus* (Moghe, 1951; Pl. V, Fig. 26) (Pl. XIII, Fig. 12).

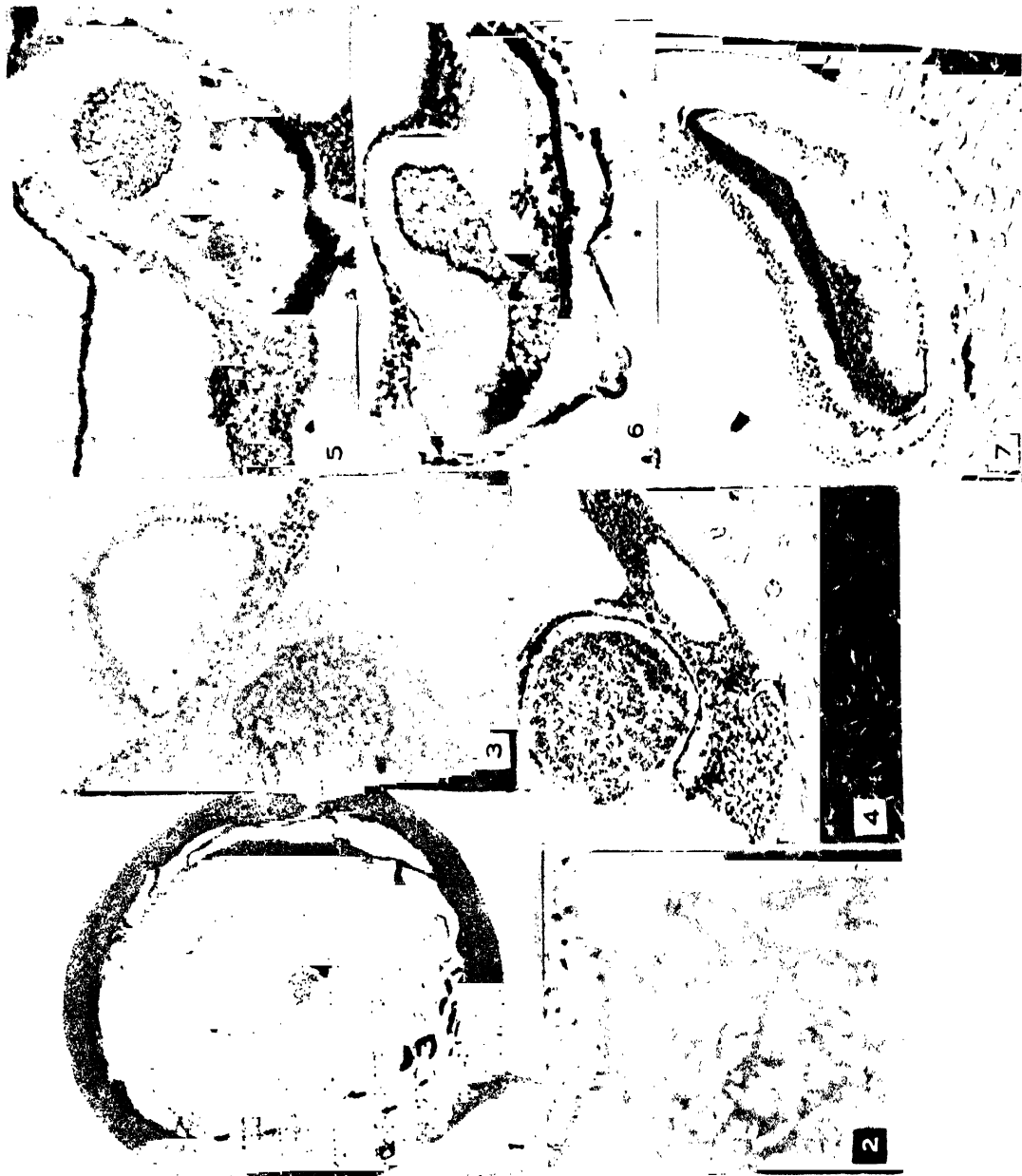
The chorio-allantoic placenta is now discoidal, the ends of the horse-shoe are considerably shortened and the middle portion is thicker. The trophoblastic tubules are thin and are more closely branched. The final placenta is, therefore, discoidal, labyrinthine and haemochorial (Pl. XIII, Fig. 13).

DISCUSSION

I shall confine my remarks only to those features in the development of *Cynopterus sphinx gangeticus* which are peculiar to it or which differ from *Pteropus* and other Megachiroptera.

(1) *Implantation*.—In the absence of very early stages of development, I am unable to describe accurately the condition of early implantation. But in Blastocyst I, which is the earliest stage I possess, the implantation appears to be superficial. This statement very largely depends on the interpretation of the space on the antimesometrial and lateral sides (Pl. XII, Fig. 1). If the space represents the uterine lumen then the implantation is not superficial but interstitial. But if the space represents the degenerated endometrial region, then the implantation will have to be regarded as superficial. In my opinion the latter represents more correctly the nature of this space which is filled at several places by degenerating uterine glands.

Keibel's (1922) figures (Schema A and B, p. 530) show that the implantation in *Cynopterus marginatus* is circumferential and superficial. From some of his figures, however, I am inclined to conclude that the implantation is interstitial (Schema G and Fig. 8, pp. 539 and 542 respectively). Keibel calls the space on the antimesometrial and lateral sides as a *split* formed by the separation of a portion from the rest of the uterine wall. If this slit is not the uterine lumen then the implantation is superficial. The stage represented by Blastocyst I corresponds probably to this stage described by Keibel, in which case the implantation in *Cynopterus sphinx gangeticus* is also superficial. I am inclined to agree with Keibel regarding the nature of the space between the placental shell and the uterine wall on the antimesometrial and lateral sides.





(2) *Endoderm-mesoderm tissue*.—The occurrence of a spongy tissue above the embryonic knob and in its immediate vicinity in the extra-embryonic region is an interesting feature. This tissue is present in Blastocyst I (Pl. XII, Fig. 1). It is at first confined to the region above the embryonic knob and on both sides of the embryonic knob, and is apposed to the trophoblast. Later it becomes more abundant (Pl. XII, Fig. 4) and spreads laterally along the yolk-sac wall between the endoderm and the trophoblast. It persists up to the formation of the mesoderm. The interpretation of this tissue is difficult. It is not present in *Pteropus* or in *Cynopterus marginatus* or in the Microchiroptera.

Kohlbrugge (1913) describes in *Xantharpya amplexicaudata* the presence of a cell membrane between the embryonic knob and the trophoblast on the mesometrial side. He calls this the 'Dorsal Cap'. It is present in very early blastocysts (*vide* Kohlbrugge's Figs. 26, 27, 28 and 29). Though he does not state precisely the source of these cells, he suggests that they may be derived either from the embryonic knob or from the 'Nabel Blase'. These cells, in his opinion, contribute finally to the formation of the mesoblast. From a comparison of Kohlbrugge's figures with Pl. XII, Figs. 1, 3 and 4, I find that the cells of the 'dorsal cap' are the same as the cells which I have called 'Endoderm-mesoderm' tissue.

Such a tissue is present in the Rhesus monkey (Heusser and Streeter, 1941). They observe: 'In the 9-days (C610) (Pl. XII, Fig. 5) and slightly older stages the primary endodermal sheet is directly continuous with cells similar in appearance which extend beyond the inner cell-mass and underlie the trophoblast. As development progresses the lining cells spread even further from the embryonic mass until the stage 11 to 12 days, when the entire chorion is clothed by a thin layer of squamous cells. As to the origin of cells composing the layer, either they are the descendants of the first endodermal cells and spread to line the chorion or they arise by delamination from the trophoblast. In the younger specimen . . . the trophoblast . . . gives no evidence of being the source of the cells in question'.

The spongy tissue thus arises in the Rhesus monkey from the first endodermal cells or from the trophoblast (Heusser and Streeter, 1941) and in *Xantharpya amplexicaudata* from the embryonic knob (Kohlbrugge, 1913). I do not possess the requisite early stages which allow me to trace the origin of these cells.

(3) *Amniogenesis*.—The formation of amnion is a very peculiar and interesting feature. The primary amniotic cavity is formed by cavitation (Pl. XII, Figs. 3 and 4) as in other Megachiroptera. But, later, folds arise from the embryonic ectoderm and close over on the dorsal side to form another cavity (Pl. XII, Fig. 5)—the 'secondary amniotic cavity'. The two cavities are not in continuation with each other being separated by the roof of the primitive amniotic cavity. This, however, soon disappears and the two cavities become continuous with each other (Pl. XII, Fig. 6).

The formation of the secondary amniotic cavity by the formation of folds is a feature unknown in Megachiroptera so far studied. Keibel, however, states—'Das Ei von *Cynopterus marginatus* 11 zeigt als Besonderheit zwei mit Detritus gefüllte in Embryonalgebilde' (p. 548). In the absence of any figures to illustrate this statement and in the absence of any explanation for the significance of these cavities filled with detritus I am unable to state precisely whether these are homologous to the primitive and secondary amniotic cavities as observed in *Cynopterus sphinx jamaicensis*. But the following observation of Keibel is significant: 'Die Frage, ob das Ektoderm über der "fosse amiotique" Zugrunde geht, ist von van der Stricht für *V. noctula* verneint worden (Diskussion zu v. Beneden's Vortrag, 1889). Für *Miniopterus schreibersii* gibt Da Costa (1920) neuerdings an, dass die im Embryonalknoten entstehende primordiale Amnion-hohle ihre dorsale wand verliert und dass so "un espace" trophoectoblastique entsteht. Erst sekundär bildet sich debutant aus den replis ectoblastiques ein definitives Amnion und eine definitive Amnion-hohle. Bei einem der untersuchten Stadien von *Cynopterus* (Cyn. 9 xi) hatte ich auch den Eindruck, dass die ursprüngliche Decke des Amnion, wenigstens teilweise gesch-

wunden war, doch ist der Zustand des Prapartes so schlecht, dass ich keinen besonderen wert auf es legen kann' (p. 552).

It is thus certain that in *Cynopterus marginatus* and *C. sphinx gangeticus* in addition to the primary amniotic cavity there is formed another cavity and these two cavities later become continuous giving rise to the definitive amniotic cavity.

The methods of amnion formation in the Chiroptera have been described by Da Costa (1920) and in all mammals by Parkes (1951). But the method of the formation of amnion as is described above is a peculiar one.

Mossman suggests 'the type of amnion formation is correlated with the method of implantation . . . that in superficial implantation amniogenesis by folds is constant, but in the interstitially implanted type . . . it tends to be by cavitation' (quoted from Parkes, 1952, p. 100). Such a correlation seems to exist within the group Microchiroptera. Amniogenesis is by folding in *Vespertilio murinus*, *Molossus rufus*, *Nyctinomus cynocephala*. In *Vespertilio noctula* and in the Phyllostomid bats the implantation is interstitial and the amniogenesis is by cavitation. Such a correlation would also seem to exist in Megachiroptera. In all the species of Megachiroptera the implantation is interstitial and the amniogenesis is by cavitation. In *C. sphinx gangeticus* and probably in *C. marginatus* implantation is superficial and the amniogenesis is by cavitation and later by folding.

(4) *Yolk-sac gland*.—The early and subsequent disposition and behaviour of the yolk-sac in *C. sphinx gangeticus* resemble those of *Pteropus giganteus giganteus* (Moghe, 1951). During early stages the yolk-sac wall is non-vascular and later it becomes vascular. After the formation and expansion of the exocoelom the vascular splanchnopleure of the yolk-sac separates from the vitelline placental site and lies below the chorio-allantoic placenta as a collapsed bag (Pl. XIII, Fig. 9) as in *Pteropus giganteus giganteus*. Its lumen is reduced and ultimately the two walls of the yolk-sac are in apposition and the lumen is completely obliterated except for the slit-like spaces. It is now in the form of a gland which lies on the mesometrial side as in other Megachiroptera (Pl. XIII, Fig. 11). The gland itself (Pl. XIII, Fig. 12) resembles the yolk-sac gland of *Pteropus giganteus giganteus* (Moghe, 1951; Pl. V, Fig. 26). In the paper on *Pteropus giganteus giganteus* (Moghe, 1951) I have already referred to the previous literature on the subject. I also mentioned there that the conversion of the yolk-sac into a gland was a phenomenon not restricted to Megachiroptera and that similar changes take place in one of the Microchiroptera, viz. *Taphozous longimanus* (Fam. Emballonuridae) investigated by my colleague Mr. A. Gopalakrishna (MS.). Since then it has also been found in another species of Microchiroptera, *Rhinopoma kinneari* (Srivastava, 1952). Further studies on the morphogenesis, cytochemistry and histochemistry of the yolk-sac gland are in progress. I may point out here that among the two Microchiroptera in which the yolk-sac becomes a gland, the placental disc is lateral in *Taphozous longimanus* and is mesometrial in *Rhinopoma kinneari*. Mesometrial placenta is unknown in any other Microchiroptera so far studied.

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SUMMARY

1. The implantation is diffuse and appears to be superficial. The placental shell shows trophoblastic strands and syncytiotrophoblast and the uterine glands in this region have disappeared and those of the deeper region are hypertrophied. Maternal blood spaces are lined either by trophoblast or by hypertrophied endothelium in early stages of development. There is, in addition to trophoblast and endoderm, a spongy tissue both in embryonic and extra-embryonic regions. In the latter case it is between the trophoblast and the endoderm.

2. The spongy tissue becomes more abundant and spreads between the trophoblast and the endoderm in the extra-embryonic region. Spaces or clefts appear in it. This tissue disappears when the mesoderm is formed.

3. The primary amniotic cavity is formed by cavitation but there is another cavity formed on the dorsal aspect of this cavity. The two cavities are at first not contiguous, but later become so by the breakdown of the roof of the primitive amniotic cavity. The new cavity may be called the secondary amniotic cavity.

4. The yolk-sac at first has the normal disposition and structure. But in later stages, as in *Pteropus*, it becomes a shrivelled bag and lies first laterally and later mesometrially below the allantoic placental disc, and later is converted into a gland.

5. The fully formed placenta is mesometrial, discoidal, labyrinthine and haemochorial.

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EXPLANATION OF PLATES XII AND XIII

(All figures are photomicrographs. The mesometrial side is towards the bottom of figure.)

- Fig. 1 Section of the uterus with Blastocyst I (13-11-1948). There is a space outside the placental shell on the antimesometrial and lateral sides. $\times 27$.
- „ 2 A small portion of the placental shell from Fig. 1 showing basal trophoblast, cytotrophoblastic strands penetrating into syncytiotrophoblastic region. $\times 120$.
- „ 3 A magnified view of the embryonic knot showing primitive amniotic cavity, embryonic ectoderm, endoderm and spongy tissue. $\times 120$.
- „ 4 Blastocyst II (27-11-1948) showing the abundant growth of spongy tissue. $\times 120$.
- „ 5 Blastocyst III (11-11-1948) showing the primitive amniotic cavity and the secondary amniotic cavity.
- „ 6 Blastocyst IV (18-11-1948) showing the primitive amniotic cavity and the secondary amniotic cavity which are contiguous. $\times \frac{1}{2}$.
- „ 7 Prochordal stage (16-11-1948). $\times 68$.
- „ 8 Medullary groove stage (27-11-1948). The mesoderm has formed and lies between the trophoblast and the endoderm in the extra-embryonic region and has reinforced the amnion also. $\times 35$.
- „ 9. The yolk-sac is a loosely coiled bag below the placenta on the mesometrial and lateral sides. $\times 30$.
- „ 10. The structure of the placenta at the stage when the yolk-sac is gland-like. $\times 48$.
- „ 11. The lumen of the yolk-sac is obliterated and the structure resembles a gland. $\times 60$.
- „ 12. The yolk-sac gland. $\times 265$.
- „ 13. Placenta at near-term stage. $\times 56$.

AN INVESTIGATION ON THE EFFECT OF CERTAIN CHEMICALS ON THE NUCLEUS, AND THEIR POSSIBILITIES IN CHROMOSOME ANALYSIS IN PLANTS

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CONTENTS

	<i>Page</i>
1. Introduction	57
2. Materials and Methods	58
3. Observations	
(i) Treatment in Coumarin Solution	60
(ii) Treatment in Aesculin Solution	61
(iii) Treatment in Salicin Solution	61
(iv) Fragmentation Inducing Property	62
4. Discussion	62
5. Tables	65
6. Abstract	67
7. References	67
8. Explanation of Plates	68

I. INTRODUCTION

Importance of the use of chemicals affecting cell division is being more and more appreciated in recent years. The results of the investigation open up new possibilities towards solution of both academic and economic problems. The pioneer efforts of Levan and his collaborators (Tjio and Levan, 1948 ; Levan, 1949 ; Tjio and Levan, 1950) in this aspect of study provide cytologists with an extensive data on the reactivity of chromosomes to chemicals. This has been found to be a prerequisite to chromosome studies undertaken whether for purely research purpose or with a view to improving our crop plants.

A long series of chemicals, viz. phenols, alkaloids, etc., have been tested by them and their mutagenic property worked out. The cytological reactions noted after these treatments have been divided under reversible and irreversible ones. The term itself implies that the latter results in an immediate killing of the cells, whereas the former involves certain reactions conveniently grouped under narcotics. In this case the tissue naturally recovers after certain specific periods. It has been noted that, though certain chemicals are toxic even with the slightest dose applied, most of the others can induce both reversible and irreversible ones under specific concentrations.

A study of the lethal or the toxic effect naturally serves no other purpose except revealing the internal structure of the chromosomes, fully clarified. It results out of a dissolution of the matrical substance from the chromosome arms, an effect attributable possibly to the depolymerization of nucleic acid. The action no doubt, though harmful to the chromosome structure, is useful for a study of the ultimate structure of chromosomes.

On the other hand, a study of the subnarcotic and narcotic effects yields interesting data, which are of immense use not only for a study of chemical make-up of the

chromosomes, but also reveal the possibilities of their application in the improvement of plants. The lower and the upper threshold concentrations, necessary for the activities of these drugs, have been thoroughly worked out. In addition to the production of colchicine-tumours, other effects manifested fall under the category of pseudochiasma, chromosome erosion, fragmentation, translocation, etc. Chromosome erosion, a term coined by Levan, implies the appearance of innumerable beaded structures resembling secondary constrictions. By pseudochiasma is meant the adherence of chromatids at certain localized points due to difficulties in separation in anaphase, resembling a chiasma. The importance of these effects brought out in the 'Allium test' is far reaching, facilitating also an understanding of the problem of chromosome morphology.

Another aspect of study involves the use of chemicals for a clarification of karyotypes. The properties of oxyquinoline brought out through the efforts of Tjio and Levan (1950) in bringing out chromosome structure is a remarkable advance in this direction. Since then a number of chemicals had been tested and their properties worked out (Sharma and Bal, 1953; Sharma and Bhattacharjee, 1954; Sharma and Sarkar, 1955). A consistent fact emerging out from such studies is the capability of most of the mutagenic chemicals in bringing out the details of chromosome morphology under suitable concentrations. These concentrations generally lie near or below the lower threshold concentration required to effect abnormalities. Difficulties encountered for a study of the chromosome complement of a variety of plants, especially those with long chromosomes or high number, or both, necessitated a search being made to find out a suitable chemical for their study. The present report deals with three such substances, viz. coumarin, aesculin and salicin, the former two especially yielding excellent results. It was desired to work out their mutagenic property, if any, and also a study of their method of manifestation. Of these three chemicals tried, it would be noted that two of them, coumarin and salicin, have yielded considerable mutagenic property, and the other so far tried is devoid of the same. Though investigations on the chemicals are at present quite rich in data, a large group of them still remains uninvestigated.

2. MATERIALS AND METHODS

Chemicals

The three chemicals so far tried in the present investigation are coumarin, aesculin and salicin, the former two being closely related.

Coumarin.—This chemical is more scientifically known as 'ortho-coumaric acid-lactone' having the structure of an unsaturated lactone. It is a plant product, the sources being Acacias and Umbellifers, and very well known to physiologists for its remarkable growth-retarding activities.

Aesculin.—Aesculin is very closely allied to coumarin and, as a matter of fact, is a derivative of the latter and is chemically known as '6-glucosy-7-hydroxy coumarin'. It is also like coumarin a growth-retarding substance, obtained as a plant product from the horse-chestnut (*Aesculus hippocastanum*).

Salicin.—It is a glucoside of salicylic alcohol, obtained mainly from the weeping-willows (*Salix*).

Materials investigated

A large number of plants, on which the study of the chemicals were made, include both monocotyledons and dicotyledons. Among the monocotyledons, different species of *Crinum*, *Pancratium*, *Allium*, *Hymenocallis*, *Haemanthus*, *Zephyranthes*, and of the dicotyledons, *Pisum*, *Lathyrus*, *Vicia*, etc., were tried.

The plants were planted in pots in case of bulbous ones, whereas in case of species of *Pisum*, *Lathyrus*, etc., seeds were germinated in Petri dishes over sawdust. Root-tips were periodically collected for study.

Different types of treatment followed

Treatments of the materials with the chemicals were made with a view to make temporary as well as permanent preparations. Possibilities were investigated of making paraffin blocks for permanent preparations and methods of squashing root-tips with uniform pressure for both permanent and temporary preparations. After a series of trials the latter method was found to yield preparations much better in quality, as regards scattering and clarity of morphology of the chromosomes. Two methods were worked out for preparing permanent mounts from smeared preparations, which were as follows :—

- (a) staining smeared tissue with Feulgen solution,
- (b) staining the tissue in aceto-orcein-HCl mixture followed by smearing in orcein solution.

Treatments in all cases were made on plants with high and low number of chromosomes. As regards time and temperature required to bring out best results, treatments of different plants revealed different cardinal points. Concentration of the chemical also proved to be an important factor. Another aspect of study involved effects of mixtures of chemicals in different proportions.

In the study of fragmentation inducing property, recovery experiments were set up. These consist of treatments of root-tips intact in the plant for a desired period at a desired temperature and then allowing them to grow under normal conditions. The nuclear changes due to the chemical could be observed every day after acetic alcohol (1 : 1) fixation, followed by Feulgen squashes of the root-tips.

Procedure

The first step involved the preparation of an aqueous solution of the chemical. Most of them were found to be sparingly soluble in water. In practice, a saturated stock solution was prepared from which suitable concentrations could be obtained by dilution.

The root-tips were treated in the chemical and kept within a refrigerator or at room temperature, as the case might be, for a definite period. These were subsequently stained and hydrolysed in a mixture of 2% aceto-orcein and N.HCl (9 : 1) and held over a flame for a few seconds. Squashes were made in 1% aceto-orcein solution applying considerable uniform pressure over the coverslip to obtain a single layered smear, with well scattered metaphase plates. The method, however, necessitated the final blotting of the excess stain with the help of a filter paper. The materials, presenting difficulties in smearing, could be squashed by repeated heating and pressing. The slides were then sealed and could be kept for fifteen days or more. This preparation is obviously a temporary one and cannot be kept for a long time. The keeping quality of the slides depends upon the atmospheric conditions and the materials used. Storage in a moist chamber, however, makes them more resistant and even after a month or more they remain quite fresh. This type of temporary preparation could be made permanent by inverting the slides over 10% acetic acid or acetic : alcohol (1 : 1), and then running through acetic : alcohol : xylol grades, finally mounting in Canada balsam.

In the case of dicotyledonous materials, orcein failed to show any appreciable result in staining. Feulgen squashes were applied, instead of aceto-orcein, in such cases. Feulgen squashes involved hydrolysis of the root-tips in N.HCl at 60° for fifteen minutes and subsequent staining in the Feulgen's solution followed by squashing in 45% acetic acid. For permanent mounts, hydrolysed root-tips were smeared on a dry slide and inverted overnight in 70% alcohol. The slide after washing and subsequent staining was run through alcohol : xylol grades and finally mounted in Canada balsam.

For paraffin blocks, root-tips were fixed in a mixture of chromic acid 1%, formalin 10% and the chemical concerned in proportions of 1 : 1 : 1, 1 : 2 : 1, 1 : 3 : 1 respectively,

treated in suitable temperatures for a definite period and embedded in paraffin following usual procedures. 14μ thick sections were stained following Newton's crystal-violet-iodine technique, and an overnight mordanting in 1% chromic acid in case of materials fixed in higher dose of formalin became essential.

Photomicrographs were taken of suitable preparations from temporary mounts generally at a usual magnification of $\times \pm 900$ approximately, using U.C.E. compensating eyepiece of $\times 20$ or in rare cases $\times 10$ and 1.3 apochromatic objective with an aplanatic condenser of 1.4 N.A.

3. OBSERVATIONS

(i) Treatment in coumarin solution

Coumarin being slightly soluble in water, a saturated solution was obtained in dissolving 0.2 gm. of the chemical in 100 c.c. of water. Root-tips were treated in this saturated solution for different periods, of which two and a half hours' treatment gave the optimum result, scattering and differentiation of the chromosome morphology being perfect. Treatment of root-tips in cold at 10° – 15°C . yielded slightly better results. Although coumarin treatment resulted to some extent in contraction of the chromosome arms, the scattering and chromosome morphology was very marked, bringing high number of chromosomes, such as $2n = 88, 92$ or more even in the same plane of focus. The gap or secondary constrictions were rendered very conspicuous and sometimes the arms beyond the gaps moved a long distance, and were likely to be mistaken for fragments. But more critical observation revealed their relationship and sometimes the thread of the chromonema became conspicuous, bridging the two arms (Text-figures and Pl. XIV, Figs. 1–6 and 11–14).



TEXT-FIGURES.

Figs. 1 to 7. Effects of coumarin.

Fig. 1. *Hymenocallis daphne* ($2n = 70$), well clarified metaphase plate.

Figs. 2 to 5. Fragmentation in *Lathyrus sativus* with varying number of fragments given in outline.

Figs. 6 and 7. Fragmentation in *Pisum sativum*. $\times 900$ app.

Lower concentrations, such as 0.001%, 0.05%, 0.01%, 0.1%, etc., were tried specially on *Crinum* chromosomes. Three hours' treatment also produced good effect and a lessening of the effect was noted with decrease in concentration.

In case of *Pancratium* and *Crinum* (Pl. XIV, Figs. 1-3; 7-10 and Pl. XV, Fig. 22) it was observed that one hour treatment can bring out the effect, optimum at two and a half hours in 0.2% concentration. In treatments for longer periods, such as four to five hours, the chromosomes were noted to appear as pycnotic bodies scattered all over the cell. Sometimes granular appearance of the chromosomes was met with after such prolonged treatments.

By application of this chemical to root-tips of dicotyledonous plants, such as *Vicia*, *Pisum* and *Lathyrus*, well scattered plates were obtained no doubt, but most of them contained fragments, which have been dealt with here under a separate heading, namely 'Fragmentation inducing property' (Figs. 2-6; Pl. XV, Figs. 26-27).

In some materials, however, coumarin produced stickiness in the chromosomes, which was overcome by using a mixture of coumarin 0.2% and 0.002% 8-oxyquinoline (1 : 1) and treating the root-tips for two and a half hours in cold, which yielded quite satisfactory results in *Haemanthus* sp. (Pl. XV, Figs. 16-18).

Paraffin blocks were prepared after fixing *Crinum* root-tips in 1 : 1 : 1, 1 : 2 : 1, 1 : 3 : 1, chromic acid 1% : formalin 10% : coumarin 0.2% mixture and keeping in cold for one hour. But the slides were in no case better than squashes. The last mentioned proportion proved to be slightly superior to the other two.

(ii) Treatment in aesculin solution (Pl. XV, Figs. 20-22).

Aesculin is also very slightly soluble in water. Water saturates at 0.04% of the chemical. The aqueous solution emits a bluish tinge. Treatments were performed in saturated as well as in dilute solutions. The time factor in case of aesculin was very remarkable. 0.0026% solution gave very satisfactory results in *Crinum* after treatment for three to five minutes only in 10°-15°C. Saturated solution of aesculin was found to be the standard solution for application in different materials. In all cases controls in room temperature showed stickiness. The extremely short period of treatment, needed to bring out best result in case of *Crinum*, is not applicable to other materials. In general, the optimum effect in aesculin solution was obtained in thirty minutes' treatment. As in coumarin, here also the scattering was perfect and the constriction regions were very exaggerated due to differential contraction of the arms. A distinct advantage of aesculin method over coumarin technique is that in the former very little contraction is noted in comparison with that in the latter. As in coumarin, here also prolonged treatments render chromosomes practically spherical due to heavy contraction.

This chemical, showing such remarkable property of revealing karyotype in monocotyledons, proved to be of no use in dicotyledonous plants so far tried. *Pisum* root-tips were treated for five minutes, thirty minutes, one hour, two hours, two and a half hours, four hours in cold, but no considerable effect was noted. Different concentrations ranging from 0.0026% to 0.04% were tried also, but without any appreciable result. Aesculin was also applied in mixtures with 0.002% 8-oxyquinoline (1 : 1, 1 : 2, 1 : 3) and treated in cold for two and a half hours. Of these, a mixture of 0.04% aesculin and 0.002% oxyquinoline in the proportion of 1 : 3 yielded satisfactory results. Controls kept in pure oxyquinoline for the same period produced preparations of much inferior quality.

Aesculin, apart from all these, showed another interesting phenomenon in *Pancratium* and *Haemanthus* root-tips, which has been termed as 'Erosion' by Levan. The chromosomes show beaded appearance forming innumerable constrictions (Pl. XV, Fig. 22).

(iii) Treatment in salicin solution (Pl. XV, Figs. 23-25).

Salicin being readily soluble in water, no difficulty was obtained in preparing a 2% solution.

Root-tips were treated in 1% solution of salicin for three hours in cold (10° – 15° C.) and at ordinary temperature. In both the cases stickiness was obtained. 1.5% and 2% solution produced heavy fragmentation in cold.

2% salicin in 30% alcohol was applied to check its property of inducing fragmentation. But in this case any effect of the chemical could not be noted on the chromosomes. 2% solution in 5% alcohol showed stickiness. 1.5% salicin mixed with 10% alcohol (1 : 1) proved to be a failure in bringing out any scattering or good effect. Best result was obtained by using a mixture of 1.5% salicin and acetic : alcohol (1 : 1) in equal proportions in cold (10° – 15° C.). But morphology of the chromosomes was not so sharp as in coumarin or aesculin treated ones. Materials treated were species of *Crinum* and *Hymenocallis*.

(iv) Fragmentation inducing property

Of the chemicals tried, two, namely salicin and coumarin, showed mutagenic property, while aesculin did not show any such effect. It is remarkable to note that in case of coumarin, fragmentation was only noted in dicotyledonous materials. The mutagenic property of coumarin has been previously mentioned by D'Amato (1950).

Dicotyledonous materials tried were *Pisum*, *Lathyrus*, and *Vicia*. *Vicia*, however, was found to produce fragments spontaneously (Kihlman and Levan, 1951 ; Sharma and Bhattacharjee, 1954). So the study was centred around *Pisum* and *Lathyrus*.

Root-tips were treated in 0.2% coumarin solution for three to three and a half hours at 10° – 15° C. In every case, controls were kept in acetic : alcohol (1 : 1) fixation. The treated root-tips showed metaphase and prometaphase plates with one, two, three fragments (Figs. 2, 3, 4, 5, 6, and 7), the latter phase showing more plates with fragments. Controls kept at ordinary temperature also produced fragmentation.

Monocots, such as *Crinum* and *Allium*, were treated in coumarin solution for even six hours with no fragmentation effect. But the chromosomes gradually contracted to produce pycnotic bodies, and finally become granular by separation of those bodies into smaller units.

In order to trace the fate of the fragments, recovery experiments were set up. The root-tips of *Pisum* were treated in the solution keeping them intact with the seedlings for three hours in cold and then after washing in water, they were allowed to grow in Petri dishes, over moist sawdust. It was observed that on the subsequent day following treatment only 25% of the seedlings were healthy. On the second day 37% recovered to healthy condition. Root-tips of these healthy seedlings were fixed in acetic : alcohol (1 : 1) and the fragment frequency was found to be very low (*vide* Tables) as compared with the immediately treated one. This shows possibly that fragments do not persist for several cell generations.

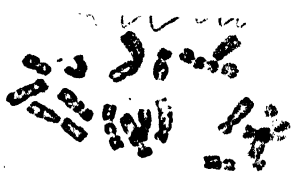
The chemical salicin was found to produce fragmentation in *Crinum* at a concentration of 1.5% and 0.2%, but a large amount of data in this direction has not yet been taken.

4. DISCUSSION

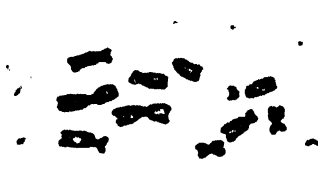
The disturbances in the cell activity up till now noted following chemical treatments have mainly been attributed to the direct change in the physical property of the cytoplasm. The change no doubt in turn affects other cell constituents and the disturbances in this activity of the chromosomes are thus to be considered as end result of the process. The nucleic acid cycle too is seriously hampered and the sticky appearance of chromosomes is claimed to be due to partial depolymerization of nucleic acid. It needs no comment that the obstruction in chromosome division and their normal activity can be caused by disturbance in the nucleic acid



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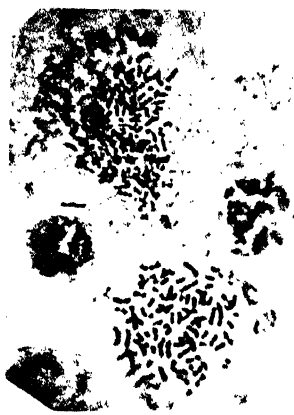
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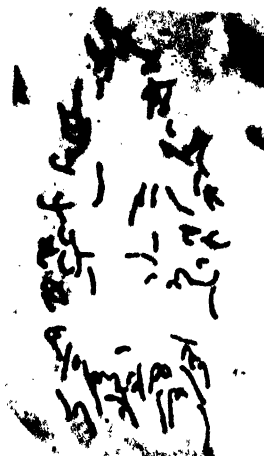
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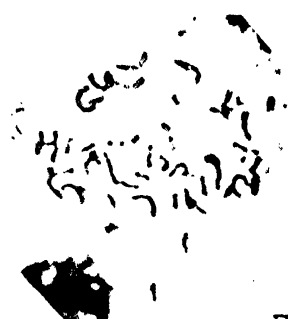
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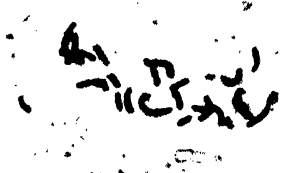
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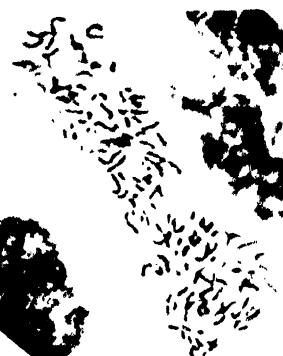
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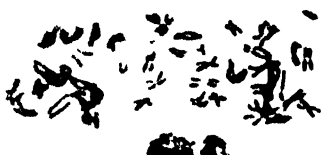
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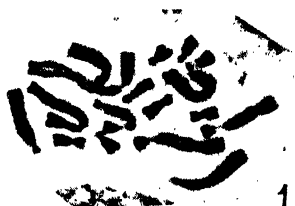
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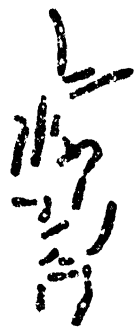
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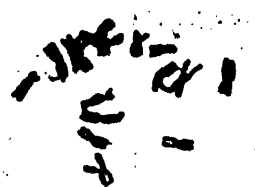
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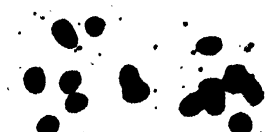
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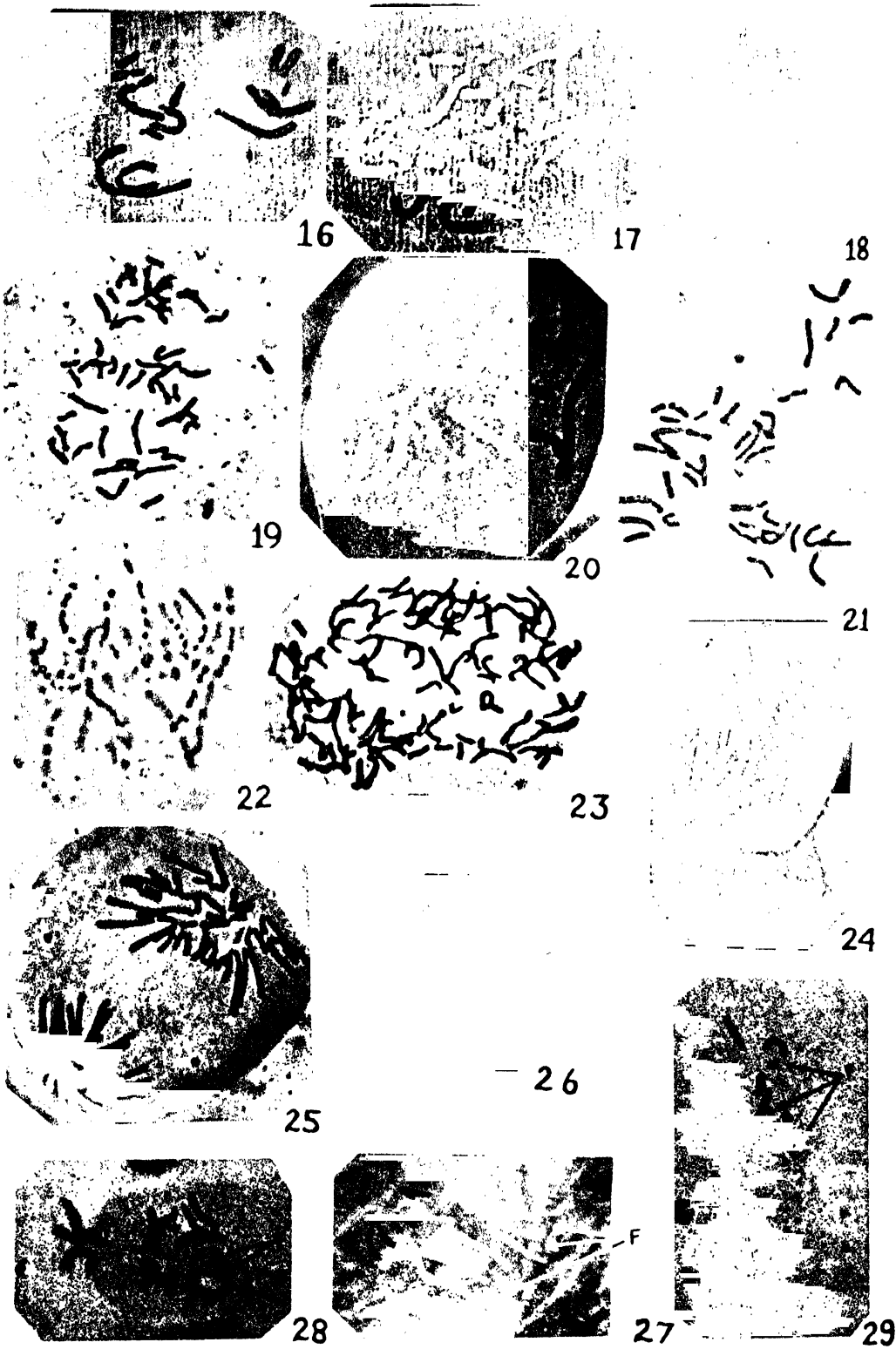
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14



15



metabolism. So far as oxyquinoline is concerned, which is mainly used for the study of karyotype, complete solidification of plasma is claimed by Stalfelt (*vide* Tjio and Levan, 1950, pp. 62-64) as the result of the effect.

The reactivity of the chromosomes towards the chemicals tried here reveals further certain interesting facts. The chemicals, though possessing the common property of affecting chromosome structure, differ in their way of attack as well as in degree.

It has been emphasized in the text that coumarin and aesculin have yielded best results in bringing out chromosome structure, salicin being the least effective one. A differential response of the chromosomes to coumarin and aesculin has further been recorded.

As far as coumarin is concerned, it has been noted that satisfactory results are obtained by using the saturated concentration of the same. In revealing chromosome structure in well scattered plates, with morphology fully clarified it possibly finds parallel in oxyquinoline. Here the effects seem to be brought about through viscosity changes in the plasma. Little difficulty is encountered in this treatment in comparison with that of oxyquinoline, as strict temperature control in cold is not found to be necessary in the former. In that way coumarin is to be considered as a much more effective chemical than oxyquinoline, so far as its effect on cell structure is concerned. Precisely, oxyquinoline is ineffective in bringing about solidification without the assistance of cold temperature, whereas the other chemical is endowed with the property of carrying out the process itself.

It is to be admitted, however, that low temperature no doubt accelerates the process even in treatments with coumarin.

The time factor in bringing about the process is remarkable and quite significant from the point of view of suitability of technique concerned. A prolonged treatment for two and a half hours to three hours has been found to be essential for oxyquinoline, whereas treatments for a lesser period are also effective in the other process. In addition to their importance in the study of karyotypes other effects have also been recorded. A discussion of these seem to be necessary for those interested in cell metabolism in general and chromosome structure in particular.

The effect needed to bring out chromosome structure in detail also involves differential contraction of chromosome arms, thus helping in an exaggerated manifestation of the constriction region. It is noteworthy that in cases where stickiness of chromosomes results in coumarin treatment possibly causing slight depolymerization of nucleic acid in certain cases, it has been observed that the difficulty can be overcome if a solution of coumarin and oxyquinoline be used in equal proportion. It seems that desoxy-ribose nucleic acid of chromosomes of such plants is liable to be depolymerized even after treatment with coumarin (this property of the chemical being not manifested in any other plant), and the effect is counteracted by the properties of oxyquinoline (Pl. XV, Figs. 16-19).

So far as the mutagenic property of coumarin is concerned, manifested in bringing about fragmentation, significant results have been obtained in different groups of plant. The saturated solution of coumarin used in the present investigation has been found to show no mutagenic effect on monocotyledonous plants, so far tried. The trials, however, included most species with long chromosome types, but a few with shorter ones have also been worked out. It is remarkable that the reactivity of monocotyledonous chromosomes to this chemical finds no parallel in the dicotyledonous ones. A prolonged coumarin treatment (3-3½ hours) in saturated solution effects fragmentation in dicotyledons, specially in the leguminous ones, tried during the present investigation. The differential behaviour of the chromatin matter towards coumarin in the two major groups of plant is significant and may in turn be a reflection of their differential composition, at least in the proportion of the constituents present. This difference might involve the cytoplasm or the chromatin matter itself or both. A detailed investigation is highly desired to find

out whether any specific regions of chromosomes are involved in this fragmentation. Though the observations so far made reveal the presence of fragments mainly in the prometaphase and prophase chromosomes, still in absence of much more confirmatory evidence under rigidly controlled conditions, it would be premature to infer as to its origin.

The fate of the fragments have also been studied following recovery experiments. Root-tips after treatment were allowed to grow and observations, carried out at intervals, reveal the decrease in the number of fragments in dividing cells. This no doubt indicates that such disturbed cells possibly fail to survive in competition with normal ones.

Prolonged treatments for more than three and a half hours in coumarin solution in monocotyledons result in extreme contraction of the chromosome arms resembling droplets embedded in plasmatic mass. The number of droplets also increase with increase in time of treatment, possibly due to transformation of the mass into smaller bodies (Pl. XIV, Fig. 15).

As regards the effect of aesculin, though excellent effects have been obtained, it must be admitted that the results so far noted are, unfortunately to some extent, erratic. The chemical, if suitably applied in proper concentration, strict timing and temperature control, is much more effective than coumarin, in the sense that the effect can be brought about in far less time. It is apparent from the test that some materials even provide brilliant plates with treatments extending only for three minutes in cold with a concentration of 0.0026% of the chemical concerned. But majority of the materials yield best result, or more precisely, the optimal effect in saturated concentration with thirty minutes' treatment. But consistent results have not been obtained in any of the material, so far as concentration and the time are concerned. It is quite likely that the physiological condition of the root-tips at the time of treatment is one of the limiting factors in the technique. A standardization of the method would naturally involve different prescriptions for root-tips collected from different soil conditions. In order to achieve this result, a series of trials following different concentrations and time on different plants, including plants from different ecological conditions, is essential.

A different way of reactivity of dicotyledonous chromosomes as observed in treatment with coumarin has also been found to some extent in aesculin too. Strictly speaking, aesculin itself is absolutely ineffective in revealing the karyotype in dicotyledons. The purpose can be served if it is applied in the form of a mixture of aesculin and oxyquinoline (1 : 3). That the effect is not due to oxyquinoline alone has also been checked from control experiments with oxyquinoline, whereby satisfactory result has not been noted. This different behaviour towards aesculin of the dicotyledonous chromosomes so far tried, thus serves an additional evidence of the possible subtle chemical difference in the composition of chromosomes of diverse groups of plants.

A constancy in the effect has been noted in all plants in continued treatment. Prolonged keeping in the fluid results in a granulation of the chromosome matter as observed in coumarin treated ones.

Of all the chemicals tried, salicin has been found to be the least effective in bringing out details of chromosome complement. Experience with other mutagenic chemicals reveals that the concentration and time needed for karyotype manifestation lies below the one required for mutagenic effect. The inability of salicin to be used as a fixative lies in the too vigorous response of the chromosome structure towards this chemical. It is unfortunate that an in-between gradient of the solution could not be found out between an ineffective concentration and one bringing about fragmentation. This might be due to some limitations of the technique whereby this suitable concentration could not be detected or the result might indicate a different mode of action of salicin towards chromosome structure, absolutely different in its fundamentals from those of aesculin and coumarin.

5. TABLES

Tables showing the occurrence of fragments in *Pisum* root-tips just after coumarin treatment.

Root-tip No. 1.

Number of fields studied	Number of dividing cells per field	Number of cells with fragments per field	Number of fragments	Stage of the dividing cell with fragment
1	3	1	1	Late prophase
2	1
3	2
4	3	2	3, 2	Late prophase, metaphase
5	6	5	2, 3, 1, 2, 3	Prometaphase 3, metaphase 2
6	3	2	2, 1	Prometaphase, prophase
7	1	1	2	Prophase
8	1
9	5	1	1	Prophase
10	2
11	4
12	4	1	1	Metaphase
13	1
14	3	1	1	Metaphase
15	1	1	1	Prophase

The frequency of dividing cells with fragments = 37.5%.

Root-tip No. 2.

Number of fields studied	Number of dividing cells per field	Number of cells with fragments per field	Number of fragments	Stage of dividing cell with fragment
1	6	1	2	Prophase
2	4
3	3	2	1, 1	Late prophase
4	2	1	1	Prophase
5	2
6	2	1	2	Metaphase
7	5	2	1, 2	Prometaphase, prophase
8	1	1	1	Metaphase
9	1
10	1
11	2
12	1	1	1	Prophase
13	2
14	1
15	2

The frequency of dividing cells with fragments = 25.6%.

Tables showing the occurrence of fragments in *Pisum* root-tips after one day recovery, subsequent to 3-3½ hours' coumarin treatment.

Root-tip No. 1.

Number of fields studied	Number of dividing cells per field	Number of cells with fragments per field	Number of fragments	Stage of dividing cell with fragment
1	6
2	2
3	4
4	1
5	1
6	2
7	3
8	2
9	1
10	2
11	2
12	4
13	4
14	2
15	2	1	1	Late prophase
16	3
17	3
18	2
19	3
20	4
21	6
22	2
23	5

The frequency of dividing cells with fragments = 1.3%.

Root-tip No. 2.

Number of fields studied	Number of dividing cells per field	Number of cells with fragments per field	Number of fragments	Stage of dividing cell with fragment
1	3
2	2
3	2
4	2
5	2
6	1
7	4
8	1
9	2
10	1
11	4
12	6
13	1
14	4
15	4
16	3
17	1
18	2
19	2
20	1
21	1
22	1
23	2
24	3
25	1

The frequency of dividing cells with fragments = 0%.

Root tip No. 3.

Number of fields studied	Number of dividing cells per field	Number of cells with fragments per field	Number of fragments	State of dividing cell with fragment
1	2
2	3	1	1	Metaphase
3	4
4	5
5	4
6	5
7	5
8	1
9	1
10	5
11	3
12	3
13	2
14	2
15	3
16	2
17	1
18	3
19	3
20	4
21	3
22	2
23	3
24	1
25	3

The frequency of dividing cells with fragments - 1.4%.

6. ABSTRACT

The present report deals with the possibility of the use of three chemicals, namely coumarin, aesculin and salicin, in chromosome analysis. On dicotyledonous and monocotyledonous plants with coumarin and aesculin successful results have been obtained. In case of aesculin, method has not yet been standardized, the treatment period varying from 3-30 minutes, but in coumarin 2-2½ hours' treatment in saturated solution in cold gives very good results. In certain cases mixture of either of them with oxyquinoline yields nice reproducible results.

The general schedule involves hydrolysis in aceto-orcein/HCl mixture for five to six seconds following the treatment, and followed by smearing in 1% aceto-orcein, applying uniform pressure over the material. Their possibilities in the preparation of paraffin block have also been worked out.

Their chromosome affecting property has been explored and certain concentrations of coumarin and salicin following three hours' treatment have been shown to possess the property of causing fragmentation. Drastic effects following prolonged treatment has also been recorded. The dicotyledonous and monocotyledonous plants were found to behave differently and their significance discussed. Recovery experiments have also been performed to indicate the gradual decrease in the frequency of fragments. The mode of action of these chemicals has also been suggested.

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8. EXPLANATION OF PLATES XIV AND XV

Microphotos—Effect of coumarin.

Figs. 1 to 3. *Crinum* sp., well clarified somatic metaphase with varying number of chromosomes.

„ 4 and 5. *Hymenocallis daphne*, showing 76 well clarified chromosomes and 2 nuclei with unequal chromosome numbers respectively.

FIG. 6. *H. harrisiana* ($2n = 92$), showing well clarified metaphase plate.

Effect of coumarin.

Figs. 7 to 10. *Pancreatium* sp., showing prophase contraction, clarified metaphase plate, diplochromosomes and anaphase stage with well scattered high number of chromosomes.

„ 11 to 13. *Haemanthus kalthregeri*, varying chromosome numbers in well clarified metaphase plates.

„ 14 and 15. *Allium cepa*, showing clear metaphase chromosomes, diplochromosomes and condensation following prolonged treatment respectively.

Figs. 16 to 19. Effect of coumarin-oxyquinoline mixture.

„ 16 to 18. *Haemanthus kalthregeri*, varying chromosome numbers in well clarified plates.

FIG. 19. *Hymenocallis senegambica*, 48 well scattered metaphase chromosomes.

Figs. 20 to 22. Effect of aesculin, well clarified metaphase stages of *Crinum* sp., *Pancreatium* sp. and oregon in *P. zeylanicum* respectively.

„ 23 to 25. Effect of salicin and acetic alcohol mixture, showing scattered chromosomes in early metaphase of *Hymenocallis daphne* and metaphase and anaphase stages of *Crinum* sp. respectively.

„ 26 to 29. Effect of coumarin.

„ 26 and 27. Fragmentation in *Pisum sativum*.

„ 28 and 29. Fragmentation in *Lathyrus sativus*.

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A MORPHOLOGICAL AND CYTOLOGICAL STUDY OF THE GAMETOGENESIS AND OOSPORE FORMATION IN *ALBUGO* SPECIES ON *IPOMOEA HEDERACEA*

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CONTENTS

	Page
Introduction	69
Symptoms of the disease	69
Material and methods	70
Development of antheridium and oogonium	70
General discussion	73
Abstract	75
Acknowledgements	75
References	75

INTRODUCTION

Investigations on the gametogenesis and oospore formation in species of *Albugo* by different investigators for the last fifty years have revealed a remarkable and interesting diversity of nuclear cycle occurring during the sexual process. No other member of Peronosporales has afforded such an interesting cytological picture. Even as far back as 1896 and later 1899, Wager and Stevens had shown the occurrence of simple and compound oospheres in species of *Albugo* including the critical details. In recent studies cytological details like stage of zonation, coenocentrum, receptive papilla, etc., have been given taxonomic importance and are used in the differentiation of species.

Cytological investigations on species of *Albugo* occurring on Convolvulaceae in India have been carried out by Damle (1943), Thirumalachar *et al.* (1949), Safeeulla (1952) and Safeeulla and Thirumalachar (1951). Studies on *Albugo evolvuli* var. *merremiae* Safee. and Thirumal. have shown characteristic cytological differences in the gametogenesis and oospore formation in addition to variations in spore measurements. Another species parasitizing the shoots of *Ipomoea hederacea* Jacq., inciting large gall formations, was previously referred to *Albugo evolvuli* var. *mysorensis* Safee. by Safeeulla (1952). A reinvestigation of the morphology and cytology of the spore forms revealed that the fungus should be given a separate specific rank. The name *Albugo mysorensis* Safee. and Thirumal. nom. nov. is proposed for its accommodation and the description of the fungus will be given later in this paper.

SYMPTOMS OF THE DISEASE

The fungus is widely distributed in North and South India. On the leaves, pedicels and calyx lobes of the flowers, the fungus produces numerous white sori as in other species of *Albugo*, which on rupture erupt large masses of spores. The infected leaves and flowers show hypertrophy, and the diseased portions may turn

yellowish in colour. Detailed examination has revealed that no sex organs are developed in the infected leaves and flowers.

The oogonia, antheridia and oospores are produced on the large spherical to cerebriform galls (Fig. 1) in the leaf axils, stems and petioles up to 2 cm. in diameter. Because they are not accompanied by sporangia, they have been overlooked by earlier workers or taken for structures unconnected with the *Albugo* species. The galls appear as small protuberances at first and gradually enlarge developing nodular surface. Mature galls are yellowish, measuring up to 3 cm. in diameter.

MATERIAL AND METHODS

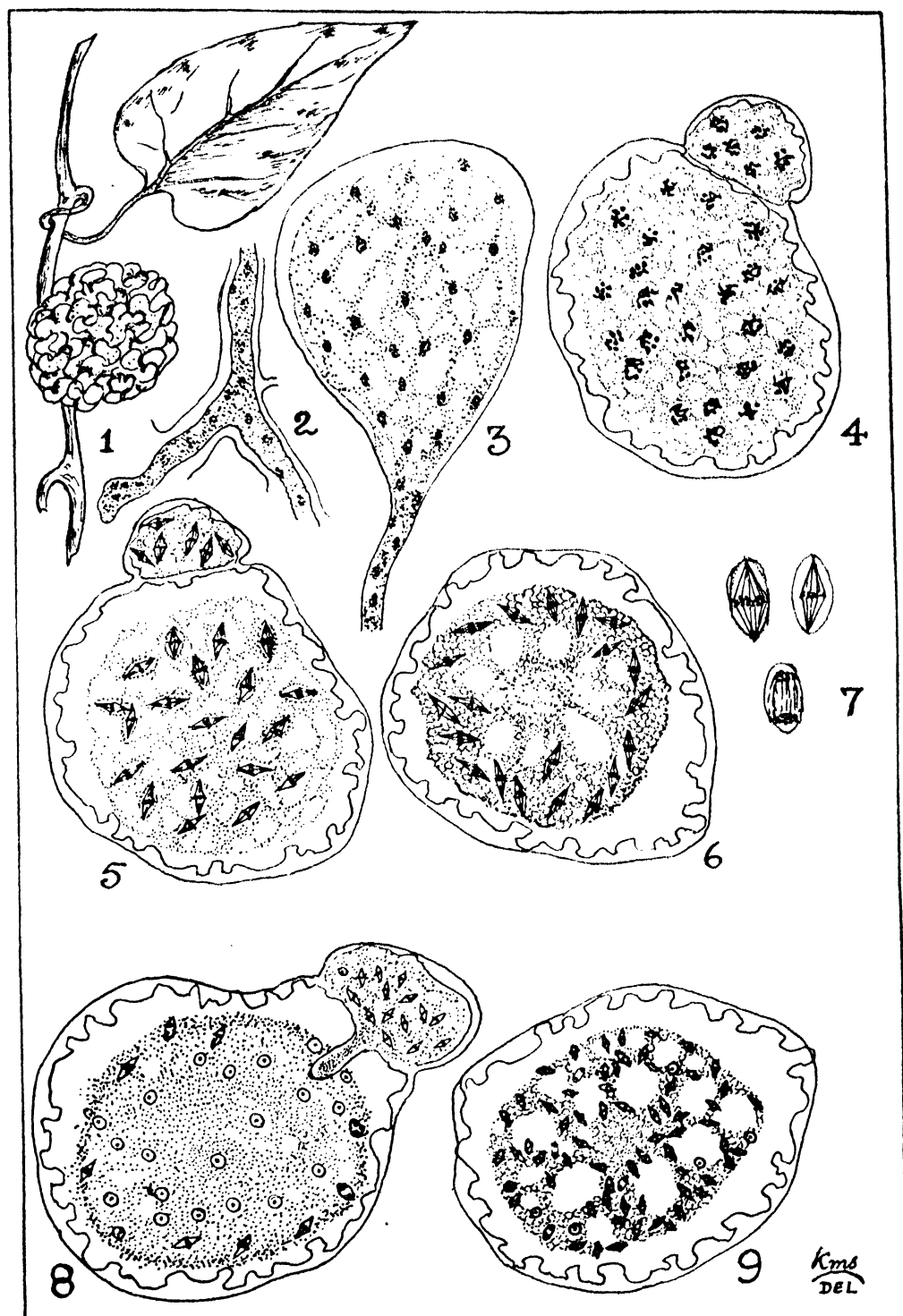
Material for cytological studies was fixed in formalin acetic alcohol or Allen's modification of Bouin's fluid. Sections of 6 to 10 μ were cut and stained with iodine gentian violet or by Heidenhain's iron-alum haematoxylin, with orange G as counter stain.

DEVELOPMENT OF ANTHERIDIUM AND OOGONIUM

The mycelium is intercellular and coenocytic containing numerous nuclei (Fig. 2), developing small haustorial processes into the host cells. The differentiation of the oogonium at the tip of the swollen hyphae (Fig. 3) is similar to that described for other *Albugo* species previously. The antheridia are paragynous and remain closely adpressed to the oogonia. Young oogonia show 30 to 40 nuclei soon after differentiation, the nuclei being distributed uniformly within cytoplasm (Fig. 4). The first division of all the nuclei within the oogonium is simultaneous with that of the antheridium (Fig. 5). The spindles of the dividing nuclei are quite prominent, intranuclear (Fig. 7), and are not arranged in a definite manner to resemble the stage of zonation reported in species like *A. bliti*, *A. portulacae* and others. After this first nuclear division, all the nuclei migrate towards the periphery and the central region is devoid of all nuclei. At this stage, there is a differentiation of well developed (Fig. 6) coenocentrum (Figs. 11 and 12) by the accumulation of the cytoplasmic material and which stains very deeply with haematoxylin. There is a second simultaneous nuclear division in the oogonium of all the nuclei most of which are redistributed again towards the central region also (Fig. 9). The second nuclear division in the oogonium may or may not be accompanied by similar nuclear divisions within the antheridium. In many cases, the second nuclear division within the antheridium precedes that in the oogonium. During the second nuclear division in the oogonium as many as 80 to 90 dividing nuclei in their metaphase stage have been counted. The differentiation of the ooplasm and periplasm takes place by the migration of a single nucleus towards the centre. The rest of the nuclei move towards the periphery and degenerate. In the degeneration process, the chromatin network in the nucleus first disappears and finally the nuclear membrane and nucleoli disintegrate. No receptive papilla has been observed.

The antheridia show at first 6 to 8 nuclei, and after the two mitotic divisions 24 to 34 male nuclei are observed within the mature antheridium. As already stated, the first mitotic division of the nuclei within the antheridium is simultaneous with that of the oogonium while the second division sometimes takes place in advance of that in the oogonium (Fig. 8).

Soon after the differentiation of the oosphere, the antheridial tube enters penetrating the periplasm (Fig. 10). A single male nucleus is discharged into the oosphere, which during the process of migration appears slightly ovate or elongated, and becomes spherical again after reaching the egg nucleus (Fig. 13). The male and female nuclei fuse immediately and the fusion nucleus is surrounded by densely staining cytoplasm (Fig. 14). A wall is laid at the region of the periplasm and the fusion nucleus undergoes free nuclear divisions. The nuclear membrane disappears prior to the differentiation of the spindles and the divisions are extra



TEXT-FIG. 1

nuclear. During the metaphase of the nuclear division of the zygote nucleus, 24 chromosomes have been counted (Figs. 15 and 16). There are 24 to 32 nuclei within the mature oospore.

The wall of the mature oospore shows two layers (Fig. 17). The endospore is smooth and coloured and the exospore is thick and slightly irregular in outline. The wall of the oogonium is persistent and is closely adpressed with the oospore wall at certain places and appearing in surface view as being covered with tuberculate projections.

GENERAL DISCUSSION

Species of *Albugo* parasitizing members of the Convolvulaceae in India include *A. evolvuli* (Damle) Safee. and Thirumal. on *Evolvulus alsinoides* L., *A. ipomoeae-aquaticae* Sawada on *Ipomoea aquatica* Forsk. (*I. reptans* Poir), *A. evolvuli* var. *merremiae* Safee. and Thirumal. on *Merremia emarginata* Hall. and present species under study referred to *A. mysorensis* on *Ipomoea hederacea*. The collection on *Ipomoea eriocarpa* from Kashmir reported by Butler and Bisby (1931) has not been available to us for examination. All the above-mentioned species are easily differentiated from *Albugo ipomoeae-panduranae* (Schw.) Swingle in the sporangial stage by the lack of the characteristic equatorial thickenings present in the wall of the sporangium. The oospores are characterized by the persistent oogonial wall which forms the outermost envelope and resembles the condition present in the genus *Sclerospora*. In contrast, the oospore in *A. ipomoeae-panduranae* resembles those of other *Albugo* species in having a large space separating the oogonial wall and the mature oospore. From these considerations, it is apparent that there is as yet no authentic record of *A. ipomoeae-panduranae* in India. The other species reported on the members of the Convolvulaceae are *A. minor* Ciferri, *A. ipomoeae-pescarpe* Ciferri, and *A. ipomoeae-hardwickii* Sawada which are characterized by the presence of equatorial thickenings of the sporangia. The types of symptoms produced on the host are characteristic of the species concerned. In *A. evolvuli* and *A. evolvuli* var. *merremiae* there is no hypertrophy of the infected portion of the host. The infection is systemic in the shoots which strangely change from the prostrate to erect habit. In *A. ipomoeae-aquaticae* the fungus is confined to portions of plant which become hypertrophied. In all the three species mentioned above, the sporangia are formed first on the plant, and from the same hyphae in later stages the oospores are formed within the host tissues. In the species on *Ipomoea hederacea* on the other hand, infection is localized and the sporangia are produced on the leaves, shoots and flowers appearing as white erumpent pustules which incite slight hypertrophy of the host. The oosporic stage produced as a result of infection from the sporangia is formed on young axillary shoots and stems. It develops at first as tiny protuberances and gradually enlarge into large cerebriform galls of considerable size. There is no covering of sporangial pustules on these galls, and these would be overlooked if one has not followed the developmental cycle of the fungus. Even very mature stages of leaf and flower-bearing sporangial infection fail to develop the oospores. The seat of sporangial and oospore development are therefore separate from each other though both belong to the same cycle. A comparative account of measurements of sporangiophores, sporangia and oospores in the four species *A. evolvuli*, *A. evolvuli* var. *merremiae*, *A. ipomoeae-aquaticae* and *A. mysorensis* is presented in Table 1. An account of the differences in cytological details are also given for the four species.

The table indicates that, apart from the characteristic differences in the type of symptoms produced on the host, and size of the sporangia and oospores, the nuclear details in the gametogenesis and oospore formation vary with respect to the number of nuclei in the oogonium and antheridium and the presence of well developed coenocentrum, etc. To indicate these differences, the allocation of a separate species for the *Albugo* species on *Ipomoea hederacea* is justified.

TABLE I

<i>Albugo evolvi</i>	<i>Albugo evolvi</i> var. <i>nerreniae</i>	<i>Albugo ipomoeae-aquaticae</i>	<i>Albugo mysorensis</i>
Infection systemic, non-hypertrophy of parts. Infection causing change from prostrate to erect habit.	Same as in <i>A. evolvi</i> .	Infection confined to portion of the shoots which are swollen.	Slight hypertrophy of sporangial infections and large oosporic galls in stems and axillary shoots.
Sex organs produced only in infected flowers and none in leaves and shoots.	Sporangia and oospores in both shoots and flowers.	Sporangia and oospores in the infected shoots.	Sporangia and oospores borne in separate infected loci.
Sporangiophores measure $37-44 \times 13-15\mu$ and sporangia $13-15 \times 11-13\mu$ without any equatorial thickening.	Sporangiophores $21-40 \times 13-17\mu$, sporangia $14-20 \times 13-18\mu$, without equatorial thickening.	Sporangiophores $27-45 \times 15-21\mu$, sporangia $18-23 \times 16-20\mu$ without equatorial thickening.	Sporangiophores $33-41 \times 13-18\mu$, sporangia $15-19 \times 13-16\mu$, without equatorial thickening.
Oogonium before organization has 30-40 nuclei.	Oogonium before organization has 30-50 nuclei.	Oogonium before organization has 30-50 nuclei.	Oogonium before organization has 80-90 nuclei.
Mature antheridium has 6-8 nuclei.	Mature antheridium has 8-16 nuclei.	Mature antheridium has 8-16 nuclei.	Mature antheridium has 24-32 nuclei.
Coenocentrum well developed and prominent.	Coenocentrum not well developed.	Coenocentrum only faintly present.	Coenocentrum very conspicuous and well developed.
Mature oospore has 8-16 nuclei.	Mature oospore has 8-16 nuclei.	Mature oospore has 8-16 nuclei.	Mature oospore has 24-32 nuclei.
Receptive papilla present.	No receptive papilla.	Receptive papilla absent.	Receptive papilla not observed.
2n number of chromosomes during meiotic division of zygote nucleus is 14-16.	2n = 16.	Number of chromosomes not known.	2n = 24.
Wall of oospore two layered, and the oogonial wall is firmly united with the outer wall of the oospore.	Oospore wall has three layers, the exospore, mesospore and endospore. The oogonial wall does not form such a firm envelope as in <i>Albugo evolvi</i> .	Wall of oospore is three layered, and the oogonial wall does form a firm envelope.	Oospore wall is two layered. The oogonial wall is confluent with the exospore only at certain points and not entirely as in <i>Albugo evolvi</i> .

Albugo mysorensis Safee. and Thirumal.

Sporangia produced on leaves, flowers and rarely on stems, inciting slight hypertrophy and paling of the leaves, white, erumpent and pulverulent. Sporangioophores subepidermal, clavate-cylindric, $33-41 \times 13-18\mu$, producing in succession chains of sporangia. Mature sporangia cuboid to spherical, without equatorial thickenings, hyaline, smooth, measuring $15-19 \times 13-16\mu$. Sex organs produced in large cerebriform galls produced by transformation of axillary buds or tender stems. Oogonia spherical, $46-58\mu$ in diameter; antheridia paragynous, measuring $16-20 \times 10-13\mu$, cinnamon-yellow to pale brown, thick-walled, with an outer exospore and inner endospore. Wall of the oogonium persistent, with the tuberculate thickenings, confluent with oospore wall at certain places, measuring $44 \times 54\mu$.

Hab. on leaves, flower and shoots of *Ipomoea hederacea*, Hebbal, Bangalore, leg. M. J. Thirumalachar (Type).

Albugo mysorensis Safee. and Thirumal, nom. nov.

Sporangia producta in foliis, floribus atque raro in culmis, inducentia, erumpentia, pulverulenta. Sporangioophori subepidermales, clavato-cylindrici, $33-41 \times 13-18\mu$, successive producentes sporangiorum catenules. Matura sporangia cuboidea vel sphaerica, absque spissatione equatoriali, hyalina, levia, magnit. $15-19 \times 13-16\mu$. Organa sexualia amplius cerebriformibus, gallae vero efformatur ex mutationem albabastrorum axillarium vel culmorum tenerum. Oogonia sphaerica, $46 \times 58\mu$ diam.; antheridia paragyna, magnit. $16-20 \times 10-13\mu$, cinnamomo-lutea vel pallide brunnea, crassis parietibus praedita; parietes vero constant exosporio externo et endosporio. Oogonii parietes persistentes vero constant exosporio externo et interno. Oogonii parietes persistentes, tuberculate spissati, confluentes cum oosporum parietibus locis denitis, magnitud. $44 \times 54\mu$.

Habitat in foliis, floribus et culmis *Ipomoeae hederaceae*, Typus lectus in loco Hebbal, Bangalore, a M. J. Thirumalachar.

ABSTRACT

A detailed morphological and cytological study of the gametogenesis and oospore formation in *Albugo* species on *Ipomoea hederacea* is made.

A comparative account of species of *Albugo* parasitizing the members of Convolvulaceae in India is presented.

Apart from the characteristic differences in the type of symptoms produced on the hosts, and size of sporangia and oospores, the nuclear details in the gametogenesis and oospore formation, an account of cytological details is also given to justify the allocation of a separate specific rank for *Albugo* species on *Ipomoea hederacea*.

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EXPLANATION OF FIGURES

Text-fig. 1. Figs. 1-9.

- FIG. 1. Showing the habit of diseased shoot, nat. size.
- .. 2. Inter cellular hypha. $\times 1,350$.
- .. 3. Oogonial initial. $\times 1,350$.
- .. 4. Oogonium and antheridium. $\times 1,350$.
- .. 5. First simultaneous nuclear division in the antheridium and oogonium. $\times 1,350$.
- .. 6. First nuclear division in the oogonium with the dividing nuclei arranged near the periphery. $\times 1,350$.
- .. 7. Intranuclear spindles $\times 2,025$.
- .. 8. Second nuclear division in the antheridium alone, prior to the division of nuclei in the oogonium. $\times 1,350$.
- .. 9. Second nuclear division in the oogonium. $\times 1,350$.

Text-fig. 2. Figs. 10-18.

- FIG. 10. Uninucleate oosphere and antheridial tube before the male nucleus is discharged. $\times 1,350$.
- .. 11. Oosphere with coenocentrum. $\times 1,350$.
- .. 12. Same as in 11, enlarged. $\times 2,025$.
- .. 13. A portion of oosphere with male and female nuclei. $\times 1,350$.
- .. 14. Oosphere with fusion nucleus. $\times 1,350$.
- .. 15. First division of the fusion nucleus. $\times 1,350$.
- .. 16. Subsequent divisions in the oospore. $\times 1,350$.
- .. 17. Mature oospore. $\times 1,350$.
- .. 18. Areole. $\times 1,350$.

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MICROFLORA AND AGE OF PUNJAB SALINE SERIES FROM DHARIALA WELL NO. 1, SALT RANGE, WEST PAKISTAN

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INTRODUCTION

Phylogenetical history of vascular plants has been a problem of great interest to biologists from time immemorial and rocks of geologically earlier formations have been the subject of continuous attack for the find of a happy solution of the question. While Lang and Cookson's (1935) and Cookson's (1935) discovery of proto-lycopod *Baragwanathia* macrofossils from the Silurian of Victoria, Australia, may be much accredited, being the first definite record of the earliest existence of vascular plants on earth, it can by no means claim to be the final clue to the problem. For reports are now gradually accumulating from different parts of the world to prove beyond doubt the occurrence of vascular flora in such an early age as Cambrian. It was Darrah who, in 1937, first recovered pteridophytic and bryophytic plant spores in the Upper Cambrian of Kolm from eastern Sweden, but as the discovery was not consistent with the prevailing conception of plant phylogeny its value was underestimated. In India, Ghosh and Bose (1947) were the first to record remnants of vascular plants in the definitely known Cambrian beds of the Salt Pseudomorph Stage, in the Punjab Salt Range, and since then they have continued examining other Cambrian beds of India, Pakistan and abroad with the result that the existence of vascular plants in Cambrian times has become more and more evident (Ghosh and Bose, 1950a, 1950b, 1952a, 1952b, 1953, 1954).

In 1953, the author received from Messrs. Burmah Oil Co., Ltd. (through Shri A. K. Ghosh) six rock specimens from different depths of Attock Oil Co.'s Dhariala Well No. 1. These specimens were from cores of the Punjab Saline Series, the top of which occurred in the well at a depth of 1,470 feet. They are representative of the Upper Gypsum/Dolomite and Salt marl stages of that Series.

DESCRIPTION OF ROCK SPECIMENS

The description of the rocks, as supplied by the Attock Oil Co., is given in the following table:—

Serial No.	Depth in Well, feet.	Description
1	1,550	Chocolate siltstone
2	1,960	Impure dolomite
3	1,987	Oil shale
4	2,350	Red marl
5	2,880	Rock salt with some inclusions of marl
6	3,965	Impure anhydrite

TECHNIQUE

Samples 1 to 4 and 6 have been macerated in solutions of equal parts of nitric and hydrochloric acid with a little quantity of potassium chlorate in addition. On dissolution of the rocks, the clayey solutions have been washed in distilled water by the process of filtration in a Buchner funnel under reduced pressure. After proper washing, the residue on the filter bed has been bleached in 10% potassium hydroxide solution for 48 hours after which thorough washing has again been done by the process already mentioned. The residue has then been added with refiltered distilled water to have the solution spread on slides, dried, and examined.

Sample 5 has been dissolved in refiltered distilled water and the solution thus formed has been examined.

Needless to say every possible precautionary measure has been taken against laboratory contamination.

OBSERVATIONS

On examination samples 3 and 4 have yielded negative results and sample 5 has given only few fibrous structures.

Microfossils of the types of woods and spores have been recovered from samples 1, 2 and 6. Some of these microfossils are described here with a view to give an idea of the nature of plants of which they form fossilized parts, and an attempt has been made to show their distribution, with reference to spores only in the Cambrian and infra-Cambrian of various regions so far examined by the author.

A. Woods.

1. Scalariform tracheid—Several tracheids with scalariform pittings recovered from samples 1 and 2. Tracheids vary from 5.5 to 6.6μ in width; rungs or thickened borders are 3.3 to 6.6μ in breadth (Pl. XVI, Fig. 1).

2. Bordered pitted tracheid—Three tracheids with remnants of adjacent ones recovered from sample 6. Tracheids are 5.5 to 11μ in width; pits bordered, compact, arranged in a row or irregularly distributed, and elliptical; size $5.5\mu \times 6.6\mu$; orifice $2.2\mu \times 5.5\mu$; borders 1.5 to 2.2μ in breadth. Some of the bordered pits occasionally widen transversely to simulate a scalariform appearance (Pl. XVI, Fig. 2).

3. Bordered pitted tracheid—Several tracheids with remnants of adjacent ones recovered from sample 1. Tracheids are 4 to 6.5μ in width. Pits bordered, round, elliptical, compact, 1-rowed or irregularly distributed; orifice 2.2μ in dimension; borders 1.5μ thick (Pl. XVI, Fig. 3).

4. Bordered pitted tracheid—A fragment of a tracheid with three round pits recovered from sample 1. Orifice $4.4\mu \times 5.5\mu$, pits crossing, $1.5\mu \times 4.4\mu$; borders 2.2μ thick (Pl. XVI, Fig. 4). Also recorded in the Salt Pseudomorph Stage (G.S.I. 57/285) in the Punjab Salt Range, and in the mid-upper Cambrian of Kashmir (G.S.I. K.32/248).

5. Pitted wood—A piece of wood with apparently simple pits recovered from samples 1, 2 and 6. Pits appear simple probably due to heavy carbonization and are separate from one another, round and 3.3μ in dimension (Pl. XVI, Fig. 5).

B. Fibres.

A fibre, $907.5\mu \times 12.1\mu$, semi-carbonized and brownish yellow in colour, recovered from sample 5. A portion of it only is shown in Pl. XVI, Fig. 6.



1



2



3



4



7



10



5



9



8



6



11



14



12



13

C. Spores.

1. Psilate spore, elliptical, $14.3\mu \times 20.9\mu$; brownish yellow in colour, wall thin (Pl. XVI, Fig. 7).

Recovered from Dharijala sample 6; also from Kolhan Series (G.S.I. 6454).

2. A round spore 18.7μ in dimension, yellow in colour, wall 1μ thick, surface appears to be rough (Pl. XVI, Fig. 8).

Recovered from Dharijala sample 1; also from Upper Kaimur, Upper Vindhya (G.S.I. 58/889), and Lower Kaimur, Upper Vindhya (G.S.I. 58/895).

3. A round spiny spore with a short flange-like structure. The entire structure is $14.3\mu \times 15.4\mu$; brownish yellow in colour; spore body $13.2\mu \times 14.3\mu$, studded with very short spines; flange 1.2μ wide, wavy, formed probably by the projecting spines (Pl. XVI, Fig. 9).

Recovered from Dharijala sample 1; also from Braintree formation, *Paradoxides harlani* zone, mid Cambrians of N. America.

4. Elliptical monoete spore, psilate, golden yellow in colour, dimension $13.2\mu \times 17.6\mu$, slit of dehiscence elongate, tapering and $1.2\mu \times 13.2\mu$ in size. Spore-wall 0.5μ thick (Pl. XVI, Fig. 10).

Recovered from Dharijala sample 2; also from Stephen formation, mid Cambrian of N. America, Upper Kaimur, Upper Vindhya (G.S.I. 58/891), and Salt Pseudomorph Stage, Punjab Salt Range (G.S.I. 57/285).

5. Elliptical spore with an elongate slit of dehiscence, golden yellow in colour, dimensions $25.3\mu \times 39.6\mu$. Slit area $16.5\mu \times 39.6\mu$; wall very thin (Pl. XVI, Fig. 11).

Recovered from Dharijala sample 1.

6. One-winged spore 45.1μ across; yellow in colour; spore body roundish, dimensions $24.2\mu \times 27.5\mu$ with a wide opening extending vertically, aperture $17.6\mu \times 22\mu$; wing densely reticulate $1\mu \times 11\mu$ in size (Pl. XVI, Fig. 12).

Recovered from Dharijala sample 1; also from Salt Pseudomorph Stage, Punjab Salt Range (G.S.I. 57/285).

7. Two-winged spore 53.9μ across; spore body deltoid, 29.7μ (vertically) \times 18.7μ (transversely, basal portion) to 39.6μ (transversely, apical portion) in size; golden yellow in colour; wings placed more or less symmetrically on two sides, reticulate, 14.3 to $20.9\mu \times 24.2$ to 29.7μ in size (Pl. XVI, Fig. 13).

Recovered from Dharijala sample 2; also from Glauconite Beds, Semri Series (G.S.I. 58/911), and Salt Pseudomorph Stage, Punjab Salt Range (G.S.I. 57/285).

8. Two-winged spore 35.2μ across; spore body round, 22μ in dimension with 5 stripes traversing the body transversely reaching the wings for a considerable distance; golden yellow in colour; wings broadly reticulate, of the same colour as the spore body and $17.6\mu \times 30.8\mu$ in size (Pl. XVI, Fig. 14).

Recovered from Dharijala sample 1; also from Glauconite Beds, Semri Series (G.S.I. 58/911), Fawn Limestones, Semri Series (G.S.I. 58/915), and Mandi Salt belt (G.S.I. 58/1032, and 58/1033).

INFERENCE

Microfossils recovered from Dharijala Well No. 1 rock samples 1 and 2 are easily comparable to those so far recorded from rocks of undisputed Cambrian age of India, Pakistan and the U.S.A. and some of the microflora recorded in sample 6 resemble those obtained from the Eparchaeon Kolhan Series.

In spite of geologists' (Christie, 1914; Fox, 1928, 1944; Gee, 1944, 1946, 1950; Pascoe, 1944; Stuart, 1919; Wynne, 1878) consistent advocacy for the Cambrian age of the Punjab Saline Series, palaeobotanists like Sahni (1944, 1946), Sitholey (1946), Lakhanpal (1946), Trivedi (1946), and others have regarded it as of Eocene or Tertiary age on the evidence of their microfossil data. The microflora so far recorded by them in different rocks of the Saline Series include various types of woods, cuticles of grass (?), cuticular structures, hairs, algal and insect remains, fungal spores,

spore-like bodies, spherical thin-walled or thick-walled spores 23.4μ to 26.4μ in dimensions, thin-walled or thick-walled ellipsoidal spores $13\mu \times 16.2\mu$ in size, spherical monolete spores 13.6μ to 26μ in dimensions, and ellipsoidal monolete spores $18.2\mu \times 31.2\mu$ in size. But it is interesting to note that similar microfossils and sometimes more advanced types of spores, e.g. trilete spores or winged spores or both, have been noted by the author in the Lower and Upper Vindhya of India, and in the Cambrian of North America, the Punjab Salt Range and Kashmir. Even in the pre-Cambrian rocks, from the Kolhan Series (G.S.I. 6454), and the Gangpur Series (G.S.I. 5013), the author has recorded the occurrence of various types of carbonized woods, e.g. scalariform, apparently simple pitted, and simple types of spores (spherical or ellipsoidal, thin-walled or thick-walled) in abundance.

These results indicate that the included plant-fossils are of no value in support of Tertiary age for the Punjab Saline Series and that they are quite consistent with the Cambrian view.

Recently Singh (1952) has reported the occurrence of trilete spores (30μ to 41μ), tricolpate pollens and pollens with germ-pores in an oil shale from the Lower Gypsum Dolomite stage of the Saline Series in the Fatehpur Maira Gorge of the Punjab Salt Range. This discovery has led him to conclude that the Saline Series is of Tertiary age.

To multiply instances of Cambrian occurrence of trilete spores, reports of the investigation by Naumova (1949), Reissinger (1938, 1952, 1953), Kopeliovitch (1951), Jacob (1949), and Jacob *et al.* (1953a, 1953b) may be mentioned. Cases of discovery of angiospermic pollens in very early beds are also not at all unknown to-day. Reissinger (1952) reports the recovery of a Potamogeton pollen in the lower Cambrian dark blue clay Kunda in Estonia; Radforth and McGregor (1954) report of their finding of a Nymphaeaceous pollen (Type G3') in the Devonian rocks from the Wabamung Lake No. 1 well. In the Carboniferous rocks, Reissinger (1952, 1953) has recovered pollens of *Iris sibirica*, *Hydrocharis morsus*, *Nymphaea*, *Nuphar*, *Polygonum*, *Potamogeton*, *Limnanthemum* and pollens resembling those of *Quercus*, *Betula*, *Carya* and other palms. These data are sufficient to indicate that the existence of angiosperms is not always characteristic of the Mesozoic or Tertiary, but that they occur also in rocks of Cambrian, of Devonian and of Carboniferous ages.

In conclusion, it may be said that since the rocks of the Dhariala well contain a microflora similar to that recovered from the undisputed Cambrian formations of the Punjab Salt Range, from the Vindhya of India and the Cambrian of Kashmir and North America, they are considered to be Cambrian in age. The Punjab Saline Series of other localities in the Salt Range also entombs microfossils most of which are identical with those found in undisputed Cambrian rocks.

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The author expresses his deep sense of gratitude to Shri A. K. Ghosh for placing the materials at his disposal and for his masterly guidance; to Dr. D. M. Bose, F.N.I., Director, Bose Institute, Calcutta, for kindly offering facilities to work in the laboratories of the Institute; to Messrs. Attock Oil Co., Ltd., for supplying the samples; to Messrs. Burmah Oil Company (India Concessions), Ltd., for providing the financial assistance, and to Shri K. L. Chowdhury of the Department of Botany, Bose Institute, Calcutta, for kindly photographing the plates.

ABSTRACT

Six rock-specimens from Cores taken from the Punjab Saline Series, Dhariala Well No. 1, have been examined. The microflora recovered resemble those recorded from undisputed Cambrian rocks. The age of the rocks analysed is, therefore, considered to be Cambrian.

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EXPLANATION OF PLATE XVI

(All the photographs are untouched)

- FIG. 1. Scalariform tracheid. $\times 450$.
" 2. Bordered pitted tracheid. $\times 450$.
" 3. Bordered pitted tracheid. $\times 450$.
" 4. Bordered pitted tracheid. $\times 1,000$.
" 5. Apparently simple pitted wood. $\times 450$.
" 6. A portion of a long fibre. $\times 450$.
" 7. Spore. $\times 1,000$.
" 8. Spore with rough surface. $\times 450$.
" 9. Spiny spore with a short flange. $\times 450$.
" 10. Monolete spore. $\times 450$.
" 11. Monolete spore. $\times 450$.
" 12. One-winged spore. $\times 450$.
" 13. Two-winged spore. $\times 450$.
" 14. Two-winged spore with stripes. $\times 450$.

Issued February 11, 1957.

ON A NEW SPECIES OF *PLATYCEPHALUS*

by L. F. DE BEAUFORT, *De Hooge Kley, Amesfoort, Netherlands*

(Communicated by B. S. Bhimachar, F.N.I.)

(Received July 9 ; approved for reading on August 3, 1956)

The genus *Platycephalus* comprises many species of bottom fishes from coastal and moderately deep waters in India and West Pacific. They have been split up into at least a dozen genera, but the general habits of all species are so alike that I prefer to keep them in one genus; also because not all groups can be clearly distinguished, for instance, a group characterized by rather large scales. Regan (1908) described three of them from Stanley Gardiner's collections in the Indian Ocean: *P. oligolepis*, *P. pedimacula* and *P. grandisquamis* from Cargados Carajos, Seychelles and Maldives, all of them from depths of 20 to 30 fathoms, commenting on these species as follows: 'The three preceding species are allied to the Japanese *P. spinosus* Schlegel and *P. macrolepis* Bleeker and differ from others of the genus in the large size of the scales'.

Max Weber collected specimens belonging to the same group during the Siboga expedition in the eastern part of the Indo-Australian Archipelago and described them (1913) as the new species of *P. macrocephalus* (S. coast of Timor, at a depth of 30 m.), and *P. grandisquamis* (off New Guinea, at a depth of 32 m.), apparently unaware of Regan's paper, for Weber's *grandisquamis* is identical with Regan's *oligolepis* and differs from *P. grandisquamis* Regan. Max Weber too laid stress on their affinity to *P. spinosus* and *P. macrolepis*.

Weber records 6 specimens of his *P. grandisquamis* (*oligolepis*), but re-examining them I found that two specimens differ considerably from the others and represent a new species which I have the pleasure to name after my friend, the late Dr. Sunder Lal Hora, F.N.I., Director, Zoological Survey of India, Calcutta.

This new species is of interest, because, although certainly belonging to the group now under consideration, it differs from the others in having the suborbital ridge not serrated and in having somewhat smaller scales, hence showing affinity to other species of the genus. This is again a proof that it is impossible to establish clear cut genera inside *Platycephalus*.

Before giving the description it will be necessary to explain some of the terms used. The bony ridges and their spines on the head of the *Platycephalidae*, and of the *Scleroparei* in general, to which order the *Platycephalidae* belong, are of taxonomic importance. I have denominated them as follows:

Supraorbital ridge on the frontal along the superior orbital rim, often continued on the parietal as superior postorbital ridge which is flanked by the inferior postorbital ridge on the preotic and ending on the post-temporal. Suborbital ridge on the suborbitals which form the characteristic 'preopercular stay' of the *Scleroparei*.

***Platycephalus horai* n. sp.**

D ¹ . VIII. D ² . 12. A. 12. P. 2. 10. 4. V. 1. 5. LI. 50. Ltr.	$\frac{2 \frac{1}{2}}{1}$ <hr style="width: 50%; margin: 0 auto;"/> 11
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Height 8.3 in length, 10.4 in length with caudal. Head 2.6 in length, 3.3 in length with caudal. Eye with a minute, simple tentacle above pupil, 3.7 in head, 1.2 in snout

and 5.5 times the concave interorbital space. Maxillary reaching to below anterior border of pupil. Bands of villiform teeth in the jaws, in two small triangular patches on the vomer, and in narrow bands on palatines. Anterior nostril with a short flap. Two spines between the anterior nostrils. One strong spine on elevated anterior orbital rim. Supraorbital ridges each with 12 or 13 crowded spines, diverging behind eyes and continued as superior postorbital ridges, which have 3-5 spines, the last one or the two posterior ones somewhat separated from the others. Anterior part of inferior postorbital ridge with 4 spines, the first immediately behind eye and more erect than the others, separated from the posterior part, which is more medial,

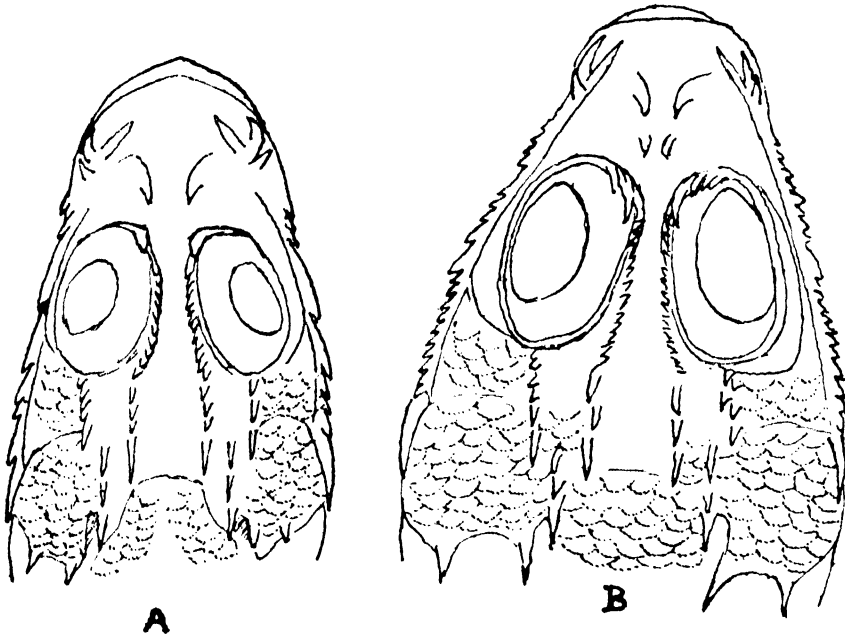


FIG. 1. Dorsal view of head of (A) *P. horai* and (B) *P. oligolepis*. $\times 4/5$.

and has three spines, the last in line with the lateral line, the two anterior scales of which have a spine. Preorbital with three diverging, flat spines, directed forward. Suborbital ridge with three small spines in front of eye, one below middle of eye, and five behind eye, the last of which at the base of the rather strong preopercular spine which is as long as pupil; below it two spines, the inferior one very small. Opercle with two strong spines. Opercle and preopercle scaly, head otherwise naked. About seven rows of scales before dorsal. First dorsal spine small, third longest, almost one-third of diameter of eye longer than postorbital part of head. Anterior longest dorsal rays as long as longest spine. Anal less deep than second dorsal. Pectorals as long as postorbital part of head. Ventrals almost as long as head without snout. Caudal slightly rounded. Scales ctenoid above, cycloid below. Colour of preserved specimens yellowish above, lighter below. Back and sides with traces of brown transverse bands, one below first dorsal more distinct. First dorsal with a broad subterminal, longitudinal band, other fins more or less spotted with dark. Length of two specimens 41 and 68 mm. Habitat: Off New Guinea, 32 m.

The relationship between this and the other species mentioned above is shown in the following key.

A. Supraorbital ridge spiny in its entire length.

- a. Suborbital ridge entirely serrated. 1 to 4 spines on anterior orbital rim.

a¹. Ll. 29-34, two anterior scales spiny *P. oligolepis*b¹. Ll. 40. Scales of anterior third of Ll. spiny *P. spinosus*

- b. Suborbital ridge with 3 spines in front of eye, one below middle of eye, and 5 behind eye. Ll. 50, the two anterior scales spiny
- P. horai*

B. Posterior part of supraorbital ridge only serrated. Sub-orbital ridge entirely serrated.

a. Four spines on anterior orbital rim. Ll. about 30 *P. pedimacula*b. One spine on anterior orbital rim. Ll. 33-36, two anterior scales spiny *P. macrocephalus*c. One or two spines on anterior orbital rim. Ll. 30, 3 or 4 anterior scales spiny *P. grandisquamis*d. One spine on anterior orbital rim. Ll. 38-40, none of the scales spiny *P. macrolepis*

SUMMARY

Fishes of the genus *Platycephalus* have similar habits. As such, no division of the fishes into genera is possible. A new species *P. horai* is described based on two specimens out of a lot labelled *P. grandisquamis* Weber.

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STUDIES ON THE DEVELOPMENT OF THE EMBRYO OF *ORYZA SATIVA* L. AND THE HOMOLOGIES OF ITS PARTS

by BISWAMBHAR SAHA*

(Communicated by G. P. Majumdar, F.N.I.)

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CONTENTS

	Page
1. Introduction	86
2. Materials and Methods	87
3. Observations	
(i) Morphology	88
(ii) Embryogeny	91
(iii) Anatomy	93
4. Discussion and Conclusion	94
5. Evidence from other Sources	98
6. Summary	98
7. Acknowledgement	99
8. References	99
9. Explanation of Figures	100

I. INTRODUCTION

During the last century and a half, the development, morphology and anatomy of the embryos and seedlings of various grasses have been extensively studied. Bruns (1892), Worsdell (1916), Avery Jr. (1930), McCall (1934), Boyd and Avery Jr. (1936), Hector (1936) and Hayward (1938), amongst others, have already reviewed relevant literature. But the homologies of different organs of the embryo have not yet been fully established.

The shield-shaped structure by means of which the grass embryos draw nourishment from the endosperm has been described since the time of Gärtner (in Goebel, 1905, p. 415) as the *scutellum*; its function is solely suctorial. The *epiblast* and the *ventral scale* are apparently outgrowths of the scutellum, a protecting device for the plumule; these are without any vascular supply. The *coleoptile* or the plumular sheath sits directly on the scutellar node and appears to arise from the latter in close association with the scutellum. The endogenous *primary root* with a *root cap* is surrounded by a peripheral tissue, the *coleorhiza*, which is continuous with the scutellum.

The most important contributions within recent years are those of Avery Jr. (1928, 1930), McCall (1934), and Boyd and Avery Jr. (1936). Mullendore's (1948) contribution is not really concerned with the homologies of these organs. Avery Jr. and Boyd and Avery Jr. advanced the views that the scutellum is the cotyledon; coleoptile, the second leaf of the plant; mesocotyl†, the first internode of the axis, and therefore, they think that the term 'mesocotyl' is a misnomer and should be discarded. McCall from a detailed study of the embryos and seedlings of wheat

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† During the germination of the seed an axis is intercalated between the scutellum and the coleoptile which was named *mesocotyl* by Čelakovský (1897—in Goebel, ii, 1905) in the seedlings of *Carex*. According to him it is neither the hypocotyl nor an internode, but a greatly elongated node (Goebel, *ibid.*, p. 412).

came to the conclusion that the epiblast is the first leaf, the scutellum is the cotyledon and the second leaf, and the coleoptile is the third leaf (perhaps a fusion of two leaves). The mesocotyl is made up of two internodes and from its three nodes spring respectively the epiblast, the scutellum and the coleoptile. Boyd and Avery Jr. have already critically examined the thesis of McCall. Souèges (1924) unlike the other investigators confined his studies to the development of the embryo of the grasses. He reported that the scutellum is the cotyledon, and the coleoptile is formed from it.

When the available literature* on the subject are taken into consideration the nature and homologies of the component parts of the grass embryo (including the mesocotyl which is not a component part of the embryo) stand thus:

<i>Scutellum</i>	..	Cotyledon; sucking apex of the cotyledon; second leaf of the plant; etc.
<i>Coleoptile</i>	..	Ligular sheath; bistipular in origin; extension of the cotyledonary sheath; foliage leaf (first of the plumule); cotyledon.
<i>Mesocotyl</i>	..	Elongated scutellar node; elongated primary node; unique structure produced by the fusion of the cotyledonary stalk with the hypocotyl; the first epicotylary internode of the axis; the first two internodes of the axis.
<i>Epiblast</i>	..	A leaf opposite the scutellum, or a little above the axis; rudimentary cotyledon† (second); an appendage of the axis or the scutellum or coleorhiza; of little or of no morphological value; together with the ventral scale it forms the cotyledonary ligule.
<i>Ventral scale</i>	..	Ligule; an outgrowth of the free limb of the scutellum.
<i>Auricle</i>	..	A pair of structures seen only in the rice embryo; marginal outgrowth of the scutellum; a part of the ventral scale.

Much of the above information is based on the studies of the embryos and seedlings of wheat, oats and maize and of other grasses. Young (1938) reported on the developmental anatomy of the seedling of the rice plant. According to him, 'the general structural make-up and the provascular system of the rice embryo closely resemble that of the oat' with slight variation. But we shall see later that our observations on the development and seedling structure of the rice embryo are substantially different from those of Young.

2. MATERIALS AND METHODS

Materials for the present studies were grown both in light and darkness in Petri dishes in the laboratory and in the Botanical Garden of Dacca University towards the middle of April, 1952 and 1953. Grains were sown at different levels of the soil. The seedlings at different stages of growth and development were fixed and preserved in FAA solution. To study the embryo in its earlier stages of germination unhusked rice grains were soaked in water for 24 and 48 hours after which they were

* Poiteau, 1809; Bruns, 1892; Čelakovský, 1897; Cannon, 1900; Goebel, ii, 1905; Coulter, 1915; Sargent and Arber, 1915; Worsdell, 1916; Weatherwax, 1920, 1923; Percival, 1921, 1927; Nishimura, 1922; Souèges, 1924; Arber, 1925, 1934; Howarth, 1927; Avery Jr., 1928, 1930; Boyd, 1930, 1931, 1932; McCall, 1934; Boyd and Avery Jr., 1936; Young, 1938; Mullendore, 1948.

† Those who believed the epiblast to be the rudimentary second cotyledon are the supporters of the theory of the origin of monocotyledony from dicotyledony by the suppression of one cotyledon. They, therefore, think that the cotyledon in monocotyledons is really lateral instead of terminal with reference to the axis.

isolated from the endosperm and killed, fixed and preserved separately. To study the development of the embryo rice grains of different stages of maturity were collected from the panicles of the rice plant and the embryos were carefully isolated and fixed for microtome sectioning.

At the time of use the preserved materials were dehydrated and embedded in paraffin according to the schedule. Most of the sections were cut 8μ and 10μ thick and stained in safranin and fast green combination.

To study the vascular system of the mesocotyl the material was cleared in absolute alcohol. The cleared mesocotyl was then washed, mounted in 50 per cent glycerine and the cortical parenchyma removed with a pair of mounted needles. The origin and course of the scutellar bundle in the stele and through the cortex to the distal end of the scutellum could be clearly traced in the transparent mesocotyl.

3. OBSERVATIONS

(i) *Morphology*

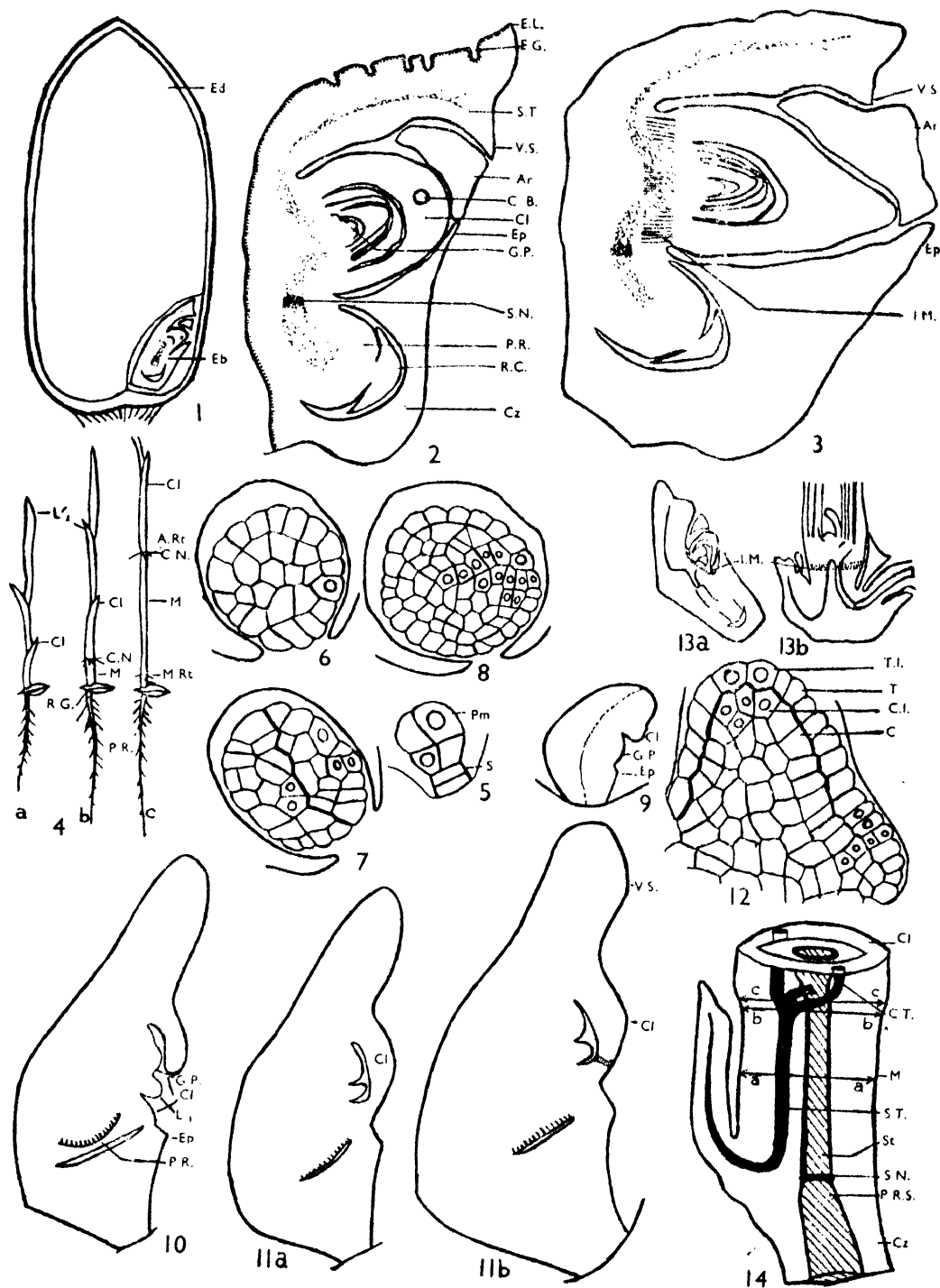
The mature grain of rice is enclosed within husks. The embryo is situated at one side toward the base of the caryopsis and lies at one side of the endosperm (Fig. 1). In longitudinal sections parallel to its wider surface the embryo is seen to consist of a short axis with the plumule at its apex and the primary root at its base. There is a short interval in the axis between the divergence of the first plumular leaf and that of the coleoptile which sits directly on the scutellum, there being no free axis between the last two. The ventral scale, a pair of auricles, epiblast, coleorhiza and the primary root with its cap complete the mature embryo of the rice plant.

The vegetative bud of the embryo or the plumule consists of a growing point surrounded by three leaf primordia, the first of which is on the side of the axis opposite the scutellum (Fig. 2). It is a cataphyll represented only by the sheathing base without the lamina. The plumule is entirely covered by the coleoptile which is a cone-shaped structure. Two large vascular bundles occur in the coleoptile one on either side in a plane parallel to the face of the scutellum. It is very thick in its terminal region but comparatively thinner on the side opposite the scutellum (Figs. 2, 32).

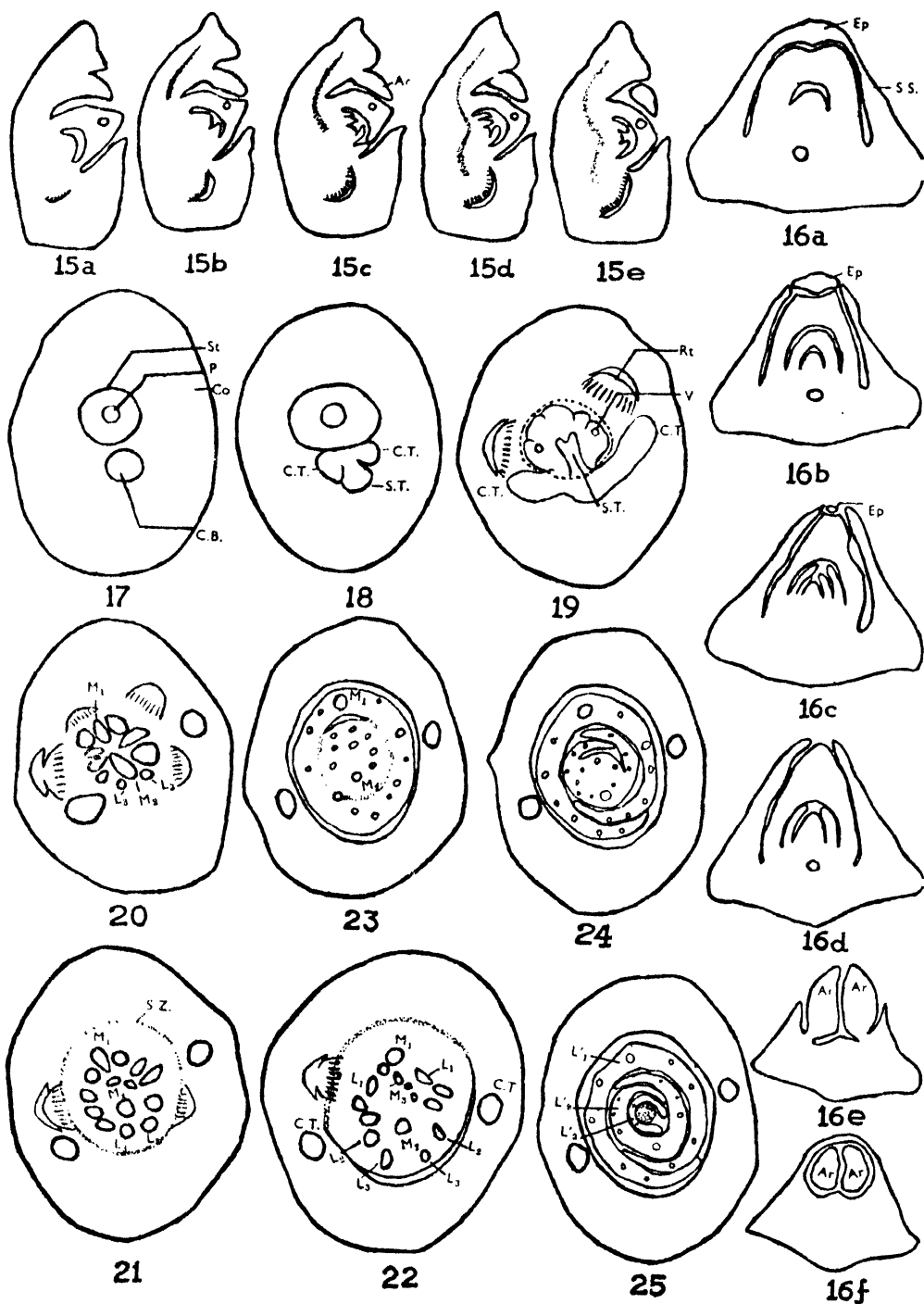
The embryonic axis is bent and during early stages of germination this bending increases to such an extent that the plumule and the radicle come to lie almost side by side (Fig. 3), with the result that the ventral scale, the auricles and the epiblast together make an efficient protective covering over the pocket containing the ensheathed plumule (Figs. 2, 3). It appears from the texture, composition and the continuity of the tissues that these three extra protective structures belong to the same organ, i.e. the scutellum.

The rice embryo, as we have already mentioned before, possesses the ventral scale, a pair of structures named in this paper, auricles, and the epiblast. The ventral scale is a small protrusion of the free terminal limb of the scutellum developed after the coleoptile. It overhangs the cavity containing the plumule, and partially covers the gap left open by the receding free margins of the scutellar sheath. A splitting of the scutellar margins results in the formation of the pair of auricles (described by Bruns, 1892, as 'marginal outgrowths of the scutellum', Goebel, ii, p. 417). The epiblast is a tongue-like outgrowth of the fused margins of the scutellar sheath on the ventral side of the embryo. Together with the ventral scale it forms an outer and the pair of auricles the inner extra-protective coverings of the plumule (Figs. 2, 3, 30-32).

The scutellum is largely parenchymatous, and has a prominent layer of epithelial cells in contact with the endosperm tissue. The epithelial cells are cylindrical and elongated with their long axes at right angles to the surface of the scutellum.



(TEXT-FIGS. 1-14)



(TEXT-FIGS. 15-25)

The upper region of this layer has infoldings to form tubular epithelial glands (Figs. 2, 30).

Its vascular system consists of a broad procambial strand of elongated thin-walled cells which originates almost vertically from the scutellar node and then follows an oblique course to enter the upper limb of the scutellum and ends near its distal end. It gives out fine branches towards the epithelial layer (Fig. 3).

The primary root is organized in the basal region of the proembryo. The root terminates in a growing point covered by a distinct root-cap. The primary root is enclosed in the root-sheath or coleorhiza which is not an identifiable structure in *Oryza*, but is merely a mass of parenchyma covering the radicle (Figs. 3, 30). The adventitious roots take their origin both in the mesocotyl and from the base of the plumule.

Germination.—Under conditions favourable for germination both plumule and radicle elongate, the coleorhiza bursts through the pericarp and is seen penetrated by the primary root, which grows rapidly downward. Soon after the emergence of the coleorhiza the coleoptile comes out with the enclosed plumule by the development of the mesocotyl between the coleoptile and the scutellum. It remains closed until its base is at level with or slightly below the surface of the soil when its edges split apart near its tip on the side opposite the scutellum to allow the plumule to pass out of it.

We have already noticed that mesocotyl is absent in the rice embryo. It is developed only during germination of the seed, and is intercalated between the coleoptile and the scutellum. The growth in length of the mesocotyl is seen to vary with the depth of the soil between its surface and the germinating grains. The deeper the grains are in the soil, within a limit, the greater is the length of the mesocotyl. Near the soil surface the mesocotyl is very short; when germinated in Petri dishes it is not developed at all (Fig. 4a). Some grains were kept for germination in a glass box in direct exposure to sunlight under a substratum when the mesocotyl was found to develop, but when some of them were kept in Petri dishes in darkness no mesocotyl developed. Thus the origin and development of this organ is seen directly related to the raising of the growing tip protected by the ensheathing coleoptile, which is conical, pointed and turgid, to the surface of the soil, and is not due to absence of light. This is accomplished by the differentiation and activity of an intercalary transverse zone of meristem organized between the coleoptile and the scutellum just above the scutellar node (Fig. 3). The growth and elongation of this organ is acropetal as the meristem remains at its top and it is suggested that the meristem also contributes to the growth and elongation of the coleoptile at the base, as the length of the coleoptile is seen to a great extent to vary directly with that of the mesocotyl. In a seedling with a very long mesocotyl the coleoptile was also seen to be very long (Figs. 4a–4c).

(ii) Embryogeny

Proembryo.—The first division of the zygote is at right angles to the long axis forming a basal cell and an apical cell. The second and third divisions occur in the apical cell and are at right angles to one another and to the plane of the first division. Thus a club-shaped proembryo consisting of four cells with a basal 2-celled suspensor is formed (Figs. 5, 26). After this the cell divisions in the proembryo do not take place in any definite sequence. The suspensor soon disappears and the proembryo becomes at first globose (Figs. 6–8) and then slightly elongated (Fig. 27).

In later stages of the globose proembryo two longitudinal halves may be distinguished on staining reactions. The left half will give rise to the dorsal side of the scutellum and the coleorhiza and the right half to the growing point, ventral half of the scutellum and the coleorhiza, coleoptile, primary root, etc. (Figs. 7–9). Even in the earlier stages of the globose proembryo the ventral side showing bigger

cells with conspicuous nuclei and active division may be distinguished (Figs. 7, 8). Slight convexity on the face of the embryo preceded by active cell divisions indicates the initiation of the apical meristem and immediately above it the primordium of the coleoptile begins to appear as a slight ridge or fold on the ventral surface of the scutellum (Figs. 9, 10). The further growth and differentiation of the coleoptile results in the formation of a deep lateral notch over the organizing growing point. It soon surrounds and encloses the stem tip as a cone-shaped structure (Figs. 11a, 11b).

Scutellum.—The upper part of the differentiating scutellum grows more or less equally in both length and width. The fully organized scutellum is curved and short (Fig. 10), developed from a meristem which is rather diffuse (Fig. 27).

Meanwhile divisions of cells in all planes result in a considerable increase in the size of the proembryo and a protoderm-like outer layer can be distinguished passing over the scutellar portion in the embryo.

The left half of the proembryo consisting of vacuolating dividing cells differentiates into the dorsal regions of the scutellum and the coleorhiza. The portion which remains in contact with the endosperm forms the dorsal side of the scutellum and the free lower portion, which sharply bends away from the endosperm, forms the coleorhiza (Figs. 11, 28-30).

The protoderm cells on the dorsal surface of the scutellum begin to elongate perpendicularly to the surface and becomes recognizable as the epithelial layer (Fig. 27). Epithelial glands, as mentioned before, are numerous towards the upper region of the scutellum (Fig. 30).

Coleoptile.—The coleoptile is initiated, as we have indicated above, on the ventral surface of the scutellum at its junction with the growing point in the form of a projection developed from the outer layer (Figs. 27, 28). The growth of the coleoptilar primordium is rapid on the side where it is initiated and it soon bends over the differentiating growing point. The primordium gradually extends tangentially around the apical dome at first as an open sheath, but the free edges soon meet and unite to form a closed structure, a conical sheath, leaving a thin weak point on the anterior side, through which the shoot apex comes out during germination (Figs. 10, 11). The tissue of the coleoptile is made up of compact parenchyma with two vascular bundles bilaterally situated.

Apical meristem.—The shoot apex meristem begins as a swelling on the ventral face of the proembryo where a higher frequency of divisions in the epidermal and hypodermal cells just below the coleoptilar projection are noticed (Figs. 9, 27). In a mature embryo the apical meristem is well organized with a uniseriate tunica enclosing a corpus (Fig. 12).

Epiblast.—The primordium of the epiblast arises soon after the initiation of the coleoptile (Fig. 9) at or just below the level described as the scutellar node (Figs. 10, 11). At first it looks like a pointed and conical tip placed at right angles to the embryonic axis. As it becomes older it bends and projects upward. It grows by an apical meristem which stains deeply (Fig. 32). The tissue of the mature epiblast is parenchymatous without any vascular tissue, and is continuous with that of the scutellar (or coleorhizal) parenchyma. Figs. 16a-16c show clearly the origin and development of the epiblast. It is an outgrowth of the region where the two margins of the scutellar sheath meet at the node and fuse, it then grows upward in the form of a tongue (Fig. 32). Its function is evidently protection of the shoot apex as it closely fits in to the space left open by the receding margins of the scutellar sheath (Figs. 2, 3 and 33).

Root meristem.—The primary root primordium begins to organize after the initiation of the first leaf primordium (Figs. 10, 11). The region going to form the primary root becomes densely stained. The root terminates in a growing point covered by a distinct root cap. The root apex exhibits four distinct histogens like that of a typical monocotyledonous plant. At the apex of the root the plerome



(Figs. 26-34)

and calyptrogen are sharply defined, and there is an intermediate layer between them which is one cell layer in thickness. The lateral members of this intermediate zone divide periclinally, and the derivatives of the inner cells function as a periblem producing cortical tissue, while the outer daughter cells become the dermatogen and form the epiblast. Thus the outstanding characteristic of the root cap is its independence in origin and structure. It also shows that the origin of the vascular system in the root is independent of that of the shoot (Fig. 30).

Coleorhiza.—The coleorhiza or the root-sheath is developed from the basal region of the proembryo. It is a solid mass of parenchyma, continuous with that of scutellum and is not distinguished before the primordium of the primary root is differentiated (Fig. 11). In this respect it can be compared with the origin of the lateral roots in the pericycle and its subsequent burrowing through the cortex which forms a sheath around the developing root. The coleorhiza grows partly by cell elongation but mostly by diffused cell divisions. The coleorhizal parenchyma cannot be distinguished from that of the scutellum. No vascular bundles are formed in this part of the embryo.

Ventral Scale.—The bulge which is seen in the distal end of the scutellum (Fig. 11b) at a later stage of embryo development projects over the depression in the form of a beak which overhangs the mouth of the cavity outside the pair of auricles (Fig. 30). It is wholly parenchymatous without any vascular tissue.

Auricles.—A pair of extra protective structures, internal to the epiblast and ventral scale, are found to originate ventrally by the splitting of the scutellar margins, designated in this paper as 'auricles' (Figs. 16d–16f, 34). These develop quite later in the developing embryo (Figs. 15a–15c), become very much thickened at the upper regions and completely fill the space outside the coleoptile and the gap between the epiblast and the ventral scale.

(iii) *Anatomy*

In transverse sections the mesocotyl shows two independent groups of vascular tissues almost throughout its length, the stele proper and what has been described by Avery in oats and wheat as 'cortical bundle' (Fig. 17). Their independent course could very well be seen in cleared mesocotyl and they could be easily separated with a pair of needles. The stele and the cortical bundle have each a sheath one layer in thickness which shows O-type wall thickening when old. This may be regarded analogous to the endodermis of roots. General orientation of the xylem elements in the stele are both root-like and transitional, but the scutellar bundle and the median of the first foliage leaf are endarch. Pith, which is present, is made up of thick-walled cells. Both tracheids and vessels are found in the cortical and the stelar bundles. Two big metaxylem vessels are placed opposite in the stele and the first pair of adventitious roots originate against these two vessels (Fig. 19).

The mesocotyl elongates acropetally by means of an intercalary meristem which always remains at the base of the coleoptile (Fig. 3). In this matter the mesocotyl differs from the epicotylary internodes, which elongate as a result of the activity of intercalary meristems near their base rather than at their top. The scutellar (cortical) bundle in the seedling axis extends upward parallel to, but does not become part of the stele until it almost reaches the level of divergence of the coleoptile (Figs. 17–20). The origin and elongation of the mesocotyl is shown diagrammatically in Figs. 13 and 14.

A little below the level of divergence of the coleoptile the scutellar bundle comes out of the stele. Its differentiation in the stele is acropetal. Immediately after leaving the stele it gives out two branches which extend first laterally and then upward into the coleoptile as its traces (Figs. 21–25), but while still within the stele and before leaving the same it sends three branches, one to form the median of the second foliage leaf and the other two to form the laterals of the first leaf of

the plumule. The scutellar bundle is, therefore, responsible while still in the stele for the supply of two laterals to the first and the median of the second leaf primordia of the plumule.

The coleoptile is structurally very simple being largely parenchymatous with only small intercellular spaces. As we have mentioned before, its bundles are two in number and they remain unbranched. Each bundle contains many xylem groups and a considerable amount of phloem tissue, and is surmounted by a thick-walled cap on its dorsal surface. A bud may be present in the axil of the coleoptile.

4. DISCUSSION AND CONCLUSION

The mature embryo is the exact replica of the adult plant (vegetative) on a miniature scale. Its different parts should, therefore, correspond with the vegetative parts of the adult plant into which it will develop. As a matter of fact the vegetative bud has been described by Bower (1935) as an *embryonic plant*. If any of its parts cannot be homologized with a corresponding part of the adult body then that part should be regarded as *sui generis*. In interpreting, therefore, the parts of the rice embryo the corresponding members of the adult body have been taken into consideration.

The Plumule.—The plumule is the vegetative shoot apex of the embryo. In the structure of its apical meristem and the number of leaf primordia it resembles the shoot apex of the adult plant (Saha, 1954).

Scutellum.—The scutellum has been identified with the cotyledon by a majority of workers on grass embryogeny. The cotyledonary nature of the scutellum, with the coleoptile as its ligule, is based on evidence afforded by its relation to the embryonic axis, its origin, vascular system and its abundant parenchyma.

Results of the developmental studies described and reported in the foregoing pages on the embryogeny of rice plant show that the cotyledon is vertical and the shoot apex lateral on it. The position of the cotyledon is, therefore, terminal on the differentiating embryo so far as the free limb of the organ is considered.

In the early stages of development the proembryo can be divided longitudinally into a dorsal and a ventral half on cytohistological basis and staining reactions of its cells. The dorsal half by diffuse cell divisions grows into a continuous structure which is distinguished and differentiated later into the dorsal side of the scutellum (in contact with the endosperm) and of the coleorhiza (free from the endosperm) indicating thereby that the two organs of the mature embryo are parts of one and the same structure. The scutellum envelops the plumule, and the coleorhiza, the radicle, when it is differentiated. The ventral half of the proembryo shows more activity and soon the coleoptile, shoot apex and the epiblast are initiated. The scutellum has epithelial layer and epithelial glands though Young (1938) reports absence of the latter in his material. The coleorhiza not being in contact with endosperm does not develop the epithelial layer.

Like an adult leaf the scutellum consists of two major parts, namely, a sheath and a limb. Both the scutellar sheath and the leaf sheath are open on the far side. Therefore, when the scutellum and an adult leaf are compared the two appear homologous.

The growing point or the shoot apex is organized at the side of the proembryo simultaneously with the initiation of the coleoptile. It soon assumes the form of a mound, the apical dome, with a single layer of tunica enclosing a corpus. The first leaf of the plumule is initiated simultaneously with the organization of the radicle apex.

Coleoptile.—Both Avery Jr., Boyd and Avery Jr. and McCall regard the coleoptile as a leaf, second or third, of the embryonic plant. Their main arguments for regarding it as an independent leaf are: it originates from a node; it is supplied with vascular tissues from the axial stele as in the case of the plumular leaves;

buds develop in its axil and root primordia (adventitious) originate a little above the coleoptilar diversion. Young supports them in full.

Čelakovský, Sargent and Arber and others, however, think that the origin of the coleoptile on the same side of the axis as the scutellum (against the distichous arrangement of leaves on the adult axis), supply of vascular tissues directly from the scutellar trace outside the stele and their number, are against the leaf-nature of the coleoptile. The primordium of the coleoptile with its vascularization does not grow like that of a leaf.

The adventitious roots in maize and other grass seedlings originate from the base of the upper internode which forms the upper part of the node below (Sharman, 1942). In rice seedlings, their origin is from the base of the upper internode, so it cannot be taken as evidence for regarding the coleoptile as a leaf.

The presence of buds in the axil of the coleoptile is not difficult to explain without any reference to the leaf-nature of the organ. Sharman (1942) has shown that in maize the bud, seen in the axil of a leaf, really belongs to the upper leaf. The leaf primordium takes its origin on one side of the axis and gradually extends tangentially along the side of the latter, and where the two wings meet on the opposite side of the axis a vegetative bud differentiates in the outer layers of the corpus (origin axial and not foliar). This has been confirmed by Hsü (1944) in *Sinocalamus Beecheyana* and by Saha (1954) in rice plant. In adult plant this bud appears cauline above the scar of the leaf when the latter is removed. Therefore, the bud, seen in the axil of the coleoptile, really belongs to the first leaf of the plumule.

Position of the coleoptile is axillary to the free limb of the scutellum and the bundles are only two, but if a leaf its origin should have been on the opposite side of the scutellum and the number of the bundles at least three in the early stage of development. The coleoptile bundles again do not come directly from the stele as reported by McCall in wheat, but they come from the scutellar trace outside the stele as its branches, showing thereby that the coleoptile, like the stipular portion of the ligule of adult leaves, is a part (outgrowth) of the scutellum.

Goebel (1905) thinks that 'the coleoptile is an outgrowth of the scutellum and it corresponds to the cotylar sheath of other monocotyledonous plants' (p. 418). He illustrates his point by drawing an example from an immature embryo of *Hordeum hexastichum* (Fig. 262, p. 418), where the coleoptile is compared to the axillary stipule. He further shows that the embryo of *Zizania aquatica*, which resembles that of *Oryza sativa*, the coleoptile is a cotylary sheath.

Hanstein (1870), however, reported that in *Brachypodium* during its (coleoptile) formation a projection is formed between the scutellum and the growing point. He also noticed another similar projection on the other side. From this McCall suggested that the coleoptile might be derived from two leaves. Avery Jr., on the other hand, could trace the origin of the coleoptile in wheat to 'the meristematic ring of cells on the lateral face of the embryonic mass of tissue which later differentiates into the scutellum. This ring of meristematic cells develops into the coleoptile'. Percival found that in the sequence of appearance the coleoptile appears next to the scutellum in the form of a ring during the development of the grass embryo. Souèges noted its development as a part of the scutellum. Goebel compares it with the axillary stipules of *Caltha palustris*.

The coleoptile of rice embryo is formed from the general region of the pro-embryo which also gives rise to the scutellum even before or simultaneously with the organization of the apical meristem. This indicates a closer relationship between the scutellum and the coleoptile. Developmentally it differs from the leaf which initiates after the apical meristem is organized.

Avery Jr. argues that if the coleoptile is a part of the cotyledon it should not have been borne at two different levels. We have already seen that the mesocotyl, which is absent in the embryo, develops only under certain conditions during

germination. In the embryo the coleoptile is directly connected with the scutellum. Therefore, its presence, noticed for the first time in the seedling, cannot be an argument against regarding the coleoptile as a part of the scutellum.

The coleoptile is an outgrowth at the base of the sheath where its primary function is to afford protection to the delicate epicotylar shoot. Both organogenically and functionally the coleoptile is, therefore, a stipule-like structure. Our studies, therefore, support Goebel who has described the coleoptile as 'the cotylar sheath' equivalent to axillary stipules (p. 418).

Mesocotyl.—Čelakovský, Van Tieghem, Goebel and others consider mesocotyl as an elongated node; McCall considers it to be made up of two internodes and Avery Jr. and Boyd and Avery Jr. consider it a single internode between the coleoptile and the scutellum. Sargent and Arber regard it as the product of fusion of the cotyledon stalk and hypocotyl, scutellum being regarded as the cotyledon.

McCall's interpretation has been criticized by Boyd and Avery Jr. (1936). We have also shown from evidences produced after 1936 (Sharman *et al.*) that the origin of the root primordia and axillary buds could be adequately explained without any reference to the origin and position of the coleoptile. Moreover, the mesocotyl shows quite a uniform mantle of cortical tissue through which passes the median bundle of the scutellum (cortical bundle) and the presence of pith in the stele of the mesocotyl is also significant.

Mitra and Majumdar (1952) have recently revived the 'mantle-core' theory of Hofmeister (1851) regarding the composition of an internode. The mantle is made up of the leaf-base or bases (cortex) which enclose a core free from vascular tissues (pith) with an exception at the nodal plexus. In monocotyledons, particularly in the grasses, the whole of the internode is made up of the lower half of the disc of leaf-insertion enclosing a core (may be hollow) which does not, perhaps, belong to the leaf. Sharman has shown this to be the case in *Zea mays* though he does not specifically mention the existence of a core apart from the disc of insertion the lower half of which elongates into the internode below the divergence of the leaf. The existence of a core has been noticed in rice stem (Saha, 1954). At the node the trace bundles of the immediate leaf and those of leaves higher up in the axis anastomose in different degrees and form horizontal plates in the nodal region which is rather vertically thick (leaf cushion). But McCall's nodes are incompletely localized in the regions where the scutellar trace bends and separates from the stele and resolves into component parts (McCall's first node), and also where the coleoptilar traces branch off to the periphery and the central region enters the stele (McCall's second node). On the above grounds the present author feels that *McCall's two-internode theory cannot be sustained*.

Boyd and Avery's one internode theory is the reiteration of the theory propounded by De Bary (1884), Coulter (1915) and Weatherwax (1923) amongst others. The whole theory is based on the assumption that the coleoptile is an independent leaf. If we have been able to prove that the coleoptile is an outgrowth of the scutellum at the base of the sheath, the theory falls through.

Could mesocotyl then be interpreted as an elongated node as suggested by Van Tieghem (1872), Čelakovský (1897), Goebel (1905), Worsdell (1916) and Bugnon (1921, 1924)? Sharman has shown that the primordium of a leaf is inserted not at one level but in the form of a disc, called the disc of insertion, equivalent to leaf cushion or the foliar foundation, i.e. *soubassement foliaires* of Grégoire (1935) and Louis (1935). The node is vertically a wider region than a mere level. In the case of *Zea mays*, as mentioned before, Sharman showed that the node is a composite structure made up of the base of the upper internode and the upper half of the disc of insertion. In maize, wheat, oats and rice embryo this node remains undisturbed, and the coleoptile and the free limb of the scutellum diverge from this node as does an adult leaf from a node. The lower half of the disc of insertion of the scutellum elongates into the internode (mesocotyl) below, the base of which

with the upper region of the radicle forms the so-called scutellar plate. We have already mentioned before that the internode is a dual structure made up of a mantle (leaf-base) and a core (axis). Therefore, the mesocotyl should be regarded as *the elongated lower half of the disc of insertion of the scutellum which encloses a central core which may be regarded as the axis. This may be considered as the hypocotyl* which according to the modern conception is made up of the base of the cotyledon and the enclosed axis. The suggestion of Sargent and Arber is partially correct if the true nature of the hypocotyl, as suggested here, is accepted.

The elongation of the mesocotyl depends on the presence of a transverse meristem across it. Its position is somewhat different in wheat, oats, maize and rice. In *Avena* and *Oryza* it is located just below the scutellar trace diversion, in *Zea* just below the coleoptilar trace diversion and in *Triticum* it occurs just below the coleoptilar node. Therefore, a meristem which is located at different regions of the mesocotyl in different plants cannot be given any phylogenetic consideration. Its function is purely a biological one. It is more so when the pattern of organization of the embryonic parts is the same in all the four cereals considered here.

McCall has studied the structure of the mesocotyl in detail. It is quite in accord with the theory outlined above. Just above the scutellar plate (at the base of the mesocotyl) where the transition between root and stem takes place, two collateral endarch bundles facing each other in the axial cylinder are noticed. One of them goes to supply the first plumular leaf as its median bundle, and the other goes to supply the scutellum, the coleoptile receiving its vascular supply from the scutellar trace outside the stele. This also shows that the scutellum and the first plumular leaf are the two successive leaves on the embryonic axis, and the coleoptile a part of the former. Other bundles of the stele do not show regular arrangement as they are all synthetic bundles made up of trace bundles of leaves higher up in the axis. They unite irregularly and show irregular disposition of xylem and phloem elements and have been mistaken for transition bundles.

Auricles.—The pair of structures described in this paper as 'auricles' are not found in other grass embryos. They originate by the splitting of the margins of the scutellar sheath which extends almost to its base. They develop enormously so as to cover all the space between the coleoptile and the ventral scale and the epiblast.

In adult leaf we also find the leaf sheath splitting to give rise to the dorsal 'sickles' and to the margins of the composite ventral ligule (Goebel, 1905; Philipson, 1935; Hector, 1936; Saha, 1952). In the rice embryo the dorsal portions of the split margins do not develop into a pair of sickles but disappear above their origin.

Bruns also noticed this pair of structures in the embryo of *Oryza*, and described them as 'marginal outgrowths of scutellum' (Goebel, ii, p. 417, Fig. 281, V) But Young's figures do not show them as separate structures apart from the ventral scale. In the mature embryo the auricles touch the ceiling of the overhanging ventral scale and appear in sections as if they have just separated from it. This fact perhaps led Young to regard them as parts of the ventral scale. Transverse sections through this region would have settled the point of their separate origin.

Ventral Scale.—Avery describes this structure as the ligule, but our present studies do not support his interpretation. Though the ventral scale and the epiblast have always been found associated together (Avery, 1930), their true morphological nature is rather difficult to suggest unless they are regarded as opportunist outgrowths of the same organ, the scutellum, as held in this paper on the results of developmental studies. Its comparatively late origin supports this view.

Epiblast.—The epiblast is a controversial structure. There is no structure corresponding to this in the adult plant. The epiblast, whenever present, has been identified either with the first leaf of the embryo, or regarded as the vestigial second cotyledon or an appendage without any morphological value, or a part of the cotylary ligule.

It cannot be the first leaf, i.e. the vestigial cotyledon (by the suppression of which monocotyledony is supposed to have evolved). It lacks vascular tissue, which is not present even as a relic. It is not universally present in grasses. It is situated below the level of the scutellar divergence and its initiation takes place quite earlier.

The present studies show that the epiblast is an outgrowth of the region where two margins of the scutellar sheath meet on the opposite side of the axis. The sheath, like the leaf-sheath of an adult leaf, is open at the anterior side and the epiblast entirely 'fills the lower portion of the gap left open on the outer side of the receding scutellar sheath'. Its upward growth is maintained by an apical meristem which organizes at the time the coleoptile is initiated.

Therefore, the ventral scale and the epiblast are both outgrowths of the scutellum, one of the free limb and the other of the scutellar sheath at the scutellar node.

Coleorhiza.—The coleorhiza is the direct downward continuation of the scutellum. A node, the scutellar node, is organized in the ventral half of the proembryo at the base of the growing point. Simultaneously with the initiation of the first plumular leaf the root apex is organized below the nodal region and it frees itself from the lower half of the proembryo in the manner a root let primordium is organized in the primary root, and then pierces or burrows through the lower end of the proembryo which appears like a sheath enclosing the radicle as the overlying cortex does in the case of an adventitious or secondary root primordium. The root cap is also organized from this tissue.

5. EVIDENCE FROM OTHER SOURCES

Goebel (ii, pp. 410–418) states that the hypogeous cotyledon of grasses comprises three regions, namely, the haustorium, the middle portion and the sheath. This he illustrates in the germinating seedlings of *Tradescantia virginica* (Fig. 273, pp. 410–411). These three regions of the cotyledon are well differentiated in the seedlings of Palm and Commelina. The same three regions are noticed in the germinating seedlings of *Carex*, *Cyperus alternifolius* and *Scirpus locustis* (Goebel, Figs. 273, 275, 277). As in the embryos of maize, oats, wheat and rice the haustorial organ (scutellum) sucks the food stored in the endosperm, the middle portion (mesocotyl) conducts the absorbed food to the growing points, and the conical sheath affords protection to the plumule during its passage through the overlying layers of soil. The scutellum, coleoptile and the mesocotyl exactly correspond to the three regions of the hypogeous cotyledon, both in position and function, cited by Goebel. Only the dual nature of the mesocotyl, scutellar base surrounding the axial core—hypocotyl, is suggested in this paper.

6. SUMMARY

Homologies of different parts of the rice embryo have been worked out. The *scutellum* is the cotyledon, and the *coleoptile* represents the axillary stipule. The *mesocotyl*, which is absent in the embryo but develops from a zone of meristem during germination, is the base or stalk of the scutellum enclosing the axis. It may be regarded as equivalent to the hypocotyl of other embryos and the use of the term mesocotyl should be continued.

The *ventral scale* and the *epiblast* are outgrowths (opportunistic growths) of the scutellar tissue, the former of its free limb and the latter of the fused margins of its sheath. The *auricles*, which are present only in the rice embryo, represent the two margins of the ligule of adult leaves. The three together afford extra protection to the plumule while passing through unfavourable environment during germination.

The *coleorhiza* seems to be the lower part of the scutellum and the *scutellar node* is a misnomer because it is really the root-stem transition plate.

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9. EXPLANATION OF FIGURES *

Text-figures have been drawn under a camera lucida. Photomicrographs have been taken only of the salient stages in the development and growth of the embryo and seedling wherever thought necessary.

Abbreviations used.—*Ar.*—auricle; *A.Rt.*—adventitious root; *C.*—corpus; *C.B.*—cortical bundle; *C.B.*—coleoptilar bundle; *C.I.*—corpus initials; *Cl.*—coleoptile; *Co.*—cortex; *Cz.*—coleorrhiza; *Eb.*—embryo; *Ed.*—endosperm; *Ep.*—epiblast; *E.L.*—epithelial layer; *E.G.*—epithelial gland; *G.P.*—growing point; *I.M.*—intercalary meristem; *L₁*, *L₂*, *L₃*—the lateral traces to the first plumular leaf; *L₁*, *L₂*, *L₃*—first, second and third plumular leaves; *M.*—mesocotyl; *M₁*, *M₂*, *M₃*—median traces to the first, second and third plumular leaves; *M.Rt.*—adventitious root from mesocotyl; *P.*—pith; *Pm.*—proembryo; *P.R.*—primary root; *P.R.S.*—primary root stele; *R.C.*—root cap; *R.G.*—rice grain; *Rt.*—root primordium; *S.*—suspensor; *S.L.*—scutellar limb; *S.N.*—scutellar node; *St.*—stele; *S.T.*—scutellar trace; *S.S.*—scutellar sheath; *S.Z.*—separation zone; *T.*—tunica; *T.I.*—tunica initials; *V.*—vessel; *V.S.*—ventral scale.

(TEXT-FIGS. 1-14)

- FIG. 1. Median longisection parallel to wider surface of the mature grain showing the size and position of the embryo in relation to endosperm. $\times 25$.
- FIGS. 2, 3. Median longisections of embryos from grains soaked in water for 24 and 48 hours. $\times 75$.
- FIG. 4. Rice seedlings grown from seeds placed at different depths of soil; Figs. 4a, 4b and 4c are respectively from surface level, three-fourths of an inch and one and a half inches deep. $\times 1$.
- FIG. 5. Proembryo at eight-celled stage with two-celled suspensor. $\times 800$.
- FIGS. 6-8. Longisections of the proembryo at successive stages of development; Figs. 7 and 8 show indications of its early differentiation into a dorsal and ventral region. $\times 800$.
- FIG. 9. Outline diagram of young embryo (l.s.) showing coleoptile, growing point and epiblast. $\times 125$.
- FIG. 10. Same at a later stage. $\times 75$.
- FIGS. 11a, b. Successive l.s. of the embryo indicating development of lateral and median portions of the coleoptile. $\times 75$.
- FIG. 12. Median l.s. of the growing point of a mature embryo; note the uniseriate tunica and a massive corpus. $\times 1020$.
- FIGS. 13a, b. Diagrammatic representations showing development of the mesocotyl from the intercalary meristem (after Young).
- FIG. 14. Reconstruction of a portion of the seedling to show vascular supply to the scutellum, coleoptile and mesocotyl.

(TEXT-FIGS. 15-25)

- FIGS. 15a-e. Serial longitudinal, and
- FIGS. 16a-f. Transverse sections of mature embryo showing the origin of the pair of auricles and the epiblast. $\times 75$.
- FIGS. 17-25. Serial transections through the mesocotyl at a-a, b-b and c-c of Fig. 14. $\times 25$.

* The magnification noted against each of the figures has been reduced to almost half its size.

PLATE XVII (PHOTOMICROGRAPHS)

(FIGS. 26-34)

- FIG. 26. Proembryo at quadrant stage with two-celled suspensor. $\times 720$.
FIG. 27. L.S. of the elongated proembryo showing development of coleoptile and growing point on its ventral side. $\times 692$.
FIG. 28. Median l.s. of embryo showing differentiation of the ventral region to give rise to the coleoptile, growing point and epiblast; the dorsal region has vacuolated cells. $\times 416$.
FIG. 29. T.S. through growing point showing encircling of the vegetative axis by the scutellum. $\times 412$.
FIG. 30. Median l.s. of the mature embryo showing epithelial glands and zonation of the apical meristem of the primary root. $\times 65$.
FIG. 31. Median l.s. of the same showing the extra-protection of the plumule afforded by the ventral scale, auricles and the epiblast. $\times 65$.
FIG. 32. Median l.s. of the mature embryo showing auricle and epiblast. $\times 115$.
FIGS. 33, 34. T.S. of the mature embryo showing the epiblast (Fig. 33) and the pair of auricles (Fig. 34). $\times 130$.

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STUDIES ON THE PHYSIOLOGY OF RICE

XI. VERNALIZATION AND DEVERNALIZATION OF WINTER AND SUMMER VARIETIES

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INTRODUCTION

Previous results from this laboratory (Sircar, 1948; Sircar and Parija, 1949; Sircar and Ghosh, 1954) indicate that the relation of germination temperature of rice and subsequent photoperiodic exposure is by no means simple. High temperature germination of summer varieties is conducive to earliness while winter varieties appeared to be temperature neutral (i.e. no effect of temperature on acceleration).

As regards the photoperiodic behaviour the main shoots of the winter varieties are very sensitive to short days but for the summer varieties short days have retarding effect and even annul the high temperature acceleration. The effect of low temperature is enhanced tiller production in winter varieties and subsequent short photoperiods induce earliness in the main shoot as well as in the tillers indicating the influence of low temperature at some stage of the development of the so-called temperature neutral winter varieties.

In rye an extremely interesting picture has been presented by Purvis and Gregory (1952) and Friend and Gregory (1953). The optimum temperature of vernalization of winter rye is about 2°–4°C. and with increase of duration vernalization effect not only increases but also it becomes stable (i.e. not to be easily devernalized at higher temperature). Friend and Gregory have shown further that higher temperature which is generally used for devernalization does accelerate flowering when it is applied for long duration after treating the seeds with low temperature.

Thus a consideration of the phenomenon of vernalization and devernalization of rye seeds emphasizes the desirability of a critical study of the temperature relations of rice seeds. Further to establish the relation between vernalization and photoperiodism in rice an extensive survey of the conditions using a wide range of temperatures and short day length is required. In the present investigation the problem has been approached by using a combination of different temperatures of germination and day length.

The effect of field temperature on flowering of a winter variety has also been studied for which sowing dates in summer and winter were used. These results are also discussed in the light of the scheme suggested by Purvis and Gregory for flowering in rye.

MATERIALS AND METHODS

This work was carried out with rice var. *Rupsail* (winter variety) and *Dhairal* (summer variety) supplied by the State Agricultural Research Station, Chinsura, West Bengal. Summer varieties are normally sown in April-May and harvested in August-September of the same year, while winter varieties are sown in seed beds in June, transplanted in July and harvested in the following December-January. The method of vernalization used is essentially the same as that described by Purvis and Gregory (1952). The air dry grains of rice were treated with Spergon (tetrachloroparabenzquinol) to prevent attacks of micro-organism and soaked in sterilized distilled water for 24 hours. Then they were transferred to sterilized Petri dishes and sprouted for 24 hours at 25°–26°C. in darkness. During subsequent treatment

the dishes were kept at temperatures $1^{\circ}\text{--}2^{\circ}\text{C}$., 12°C ., $25^{\circ}\text{--}26^{\circ}\text{C}$., $30^{\circ}\text{--}31^{\circ}\text{C}$. and 37°C . Constant water content (50% of the oven dry weight of the seed) of the sprouted grain was maintained by adding sterilized water at regular intervals. At the end of the temperature treatment all the treated seeds in Petri dishes were kept at 26°C . for 24 hours for further sprouting. The better sprouted seeds were sown in pots containing well manured garden soil. The seeds of the control sets were also soaked for 24 hours and then allowed to germinate at 26°C . for 1 day before sowing. The dates for commencing the treatments were so adjusted that all the treatments had the same sowing date and same environmental conditions during the growing period.

For photoperiodic treatment plants were grown under 8 hour short days from 8 a.m. to 4 p.m. after having natural day length above 13 hours for 53 days. The short day treatment was continued for four weeks and the plants were exposed to natural day length until flowering. During short day treatment the pots were transferred daily to a well ventilated dark room from 4 p.m. to 8 a.m.

Scoring Method:

During the growing period the detailed morphological changes of shoot apices were examined under Zeiss Sterio Binocular Microscope at regular intervals. During

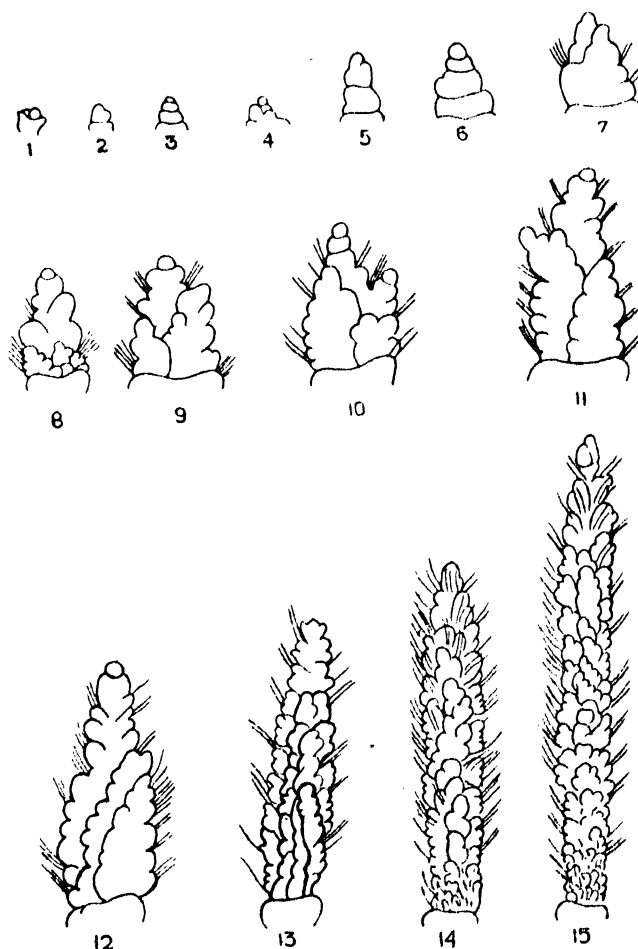


FIG. 1. Stages of the development of the shoot apex of rice var. *Rupsal*. Numbers denote the 'score'.

For explanation see the text and Table 1.

Scores Nos. 1 to 15—magnification $\times 20$.

the vegetative growth dissections were performed twice a week but after the floral initiation dissections of an average of 20 plants were made daily. To study the subsequent changes of the shoot apex during its transition from vegetative growth to flowering, the different stages of development have been carefully examined, measured and studied in several conventional units (scores). Under short day condition the rice plant after initiation of floral primordia requires approximately 22–25 days for the emergence of flower. Accordingly the stages of development of the shoot apex were divided into 25 units of scores (Figs. 1 and 2 and Table 1) and they were arranged in such a manner that each score presumably represents the daily change in the development of the shoot apex.



FIG. 2. Stages of the development of the shoot apex of rice var. *Rupsail*. Numbers denote the 'score'.

For explanation see the text and Table 1.

Scores Nos. 16–18	.. magnification $\times 10$.
Scores Nos. 19–20	.. magnification $\times 5$.
Scores Nos. 21–22	.. magnification $\times 1$.
Scores Nos. 21 (a) and 22 (a)	.. magnification $\times 5$.

EXPERIMENT 1

Vernalization and devernization of a winter variety Rupsail

This experiment was arranged to study the effect of different temperatures during germination on growth and flowering of a winter variety. The scheme of the treatment of the germinating grains was as follows:—

- (1) Continuous high or low temperature for different durations.
- (2) Alternate periods of high and low temperatures for varying durations.
- (3) Interposition of high or low temperature for a short duration in between two periods of low or high temperature for a longer duration.

The experiment was started on May 15, and seeds sown on June 15, 1954, in earthenware pots. Flowering was assessed by the date of ear emergence and score number. From the results presented in Table 2, the following facts are noticed.

TABLE I

Morphological changes in the shoot apex of rice variety Rupsail

	Size in mm.	Score
<i>Vegetative stage</i>		
Vegetative shoot apex enclosed by hood-shaped leaf	1
<i>Flowering stages</i>		
Shoot apex with globular protrusion (flower primordia) from one side of it	2
Shoot apex having two protrusions on both sides ..	0.2	3
Protrusion on all sides	0.2	4
Elongation of the apex with primary protrusion ..	0.5	5
Increase in length and breadth of primary protrusion ..	0.6	6
Transition from primary protrusion to a number of spike-lets associated with many hair-like structures ..	0.8	7
Elongation and further differentiation of the stage ..	0.9	8
Do. Do.	1.0	9
Do. Do.	1.1	10
Do. Do.	1.6	11
Do. Do.	2.0	12
Establishment of the shape of ear, ear still stiff ..	2.4	13
Increase in the length of ear, associated with grand period of extension growth of stem	3.0	14
Individual flower appears at the tip with glumes, grand period of extension growth	4.0	15
Differentiation of the individual flower from tip to base, accompanied by the elongation of the spike, grand period of extension growth	5.2	16
Do. Do.	6.0	17
Do. Do.	7.0	18
Do. Do.	13.5	19
Do. Do.	20.0	20
Grand period of elongation of ear, ear limp	82.0	21
Do. Do.	166.0	22
Emergence of the spikes from the flag leaf	171.0	23
Anthesis	24
Past anthesis	25

A continuous high temperature of germination (30°-31°C. and 37°C.) for varying durations up to 4 weeks has no direct effect on the acceleration of flower primordia over the control. These higher temperatures on the contrary delay the flowering and the delaying effect generally increases with the duration of the treatment (V_6 and V_7). Again from the Table it is evident that the scores of V_1 , V_2 , V_3 , V_4 and V_8 are less than that of the control. Similarly a continuous low temperature (i.e. 12°C.) has no effect on the acceleration of flowering rather it delays which, however, is not statistically significant (Table 2, V_{13} - V_{16}). Effects of presowing alternate temperatures show acceleration. Thus the treatments V_{17} (2 weeks at 37°C. followed by 2 weeks at 12°C.), V_{19} (1 week at 37°C. and 1 week at 12°C.), and V_{22} (alternate days at 37°C. and 12°C. for 4 weeks) induce earliness. The acceleration of V_{17} is statistically significant. But the delaying effect of alternate temperatures on other treatments can be explained by considering that the duration of temperature is important in bringing the acceleration effect. Interposition of a different temperature for 4 days in between two periods of a continuous high or low temperature is found to have no significant acceleration (V_{25} - V_{28}).

TABLE 2

Effect of presowing temperature treatments on floral initiation of Rupsail. Sowing date 15-6-1954

Treatments	Number of days for ear emergence on the main shoot (average of 10-14 plants)	Score number on 140th day of sowing (average of 10 plants)	Number of spikelets per ear Fertile/Sterile
V _c control (soaked and sprouted for 24 hrs. at 26°C.)	150.5 ± 1.7	14	100.2/16.2
V ₁ , 37°C. for 4 weeks	..	6	..
V ₂ , 37°C. for 3 weeks	..	7	..
V ₃ , 37°C. for 2 weeks	..	7	..
V ₄ , 37°C. for 1 week	..	7	..
V ₅ , 30°-31°C. for 4 weeks	..	7	..
V ₆ , 30°-31°C. for 3 weeks	163.5 ± 4.17	..	83.2/9.7
V ₇ , 30°-31°C. for 2 weeks	152.6 ± 1.2	..	86.7/10.5
V ₈ , 30°-31°C. for 1 week	..	12	..
V ₉ , 25°-26°C. for 4 weeks	..	7	..
V ₁₀ , 25°-26°C. for 3 weeks	..	8	..
V ₁₁ , 25°-26°C. for 2 weeks	157.6 ± 1.5	..	85.5/12.5
V ₁₂ , 25°-26°C. for 1 week	151.8 ± 1.6	..	88.2/7.5
V ₁₃ , 12°C. for 4 weeks	..	10	..
V ₁₄ , 12°C. for 3 weeks	154.5 ± 1.5	..	70.2/14.2
V ₁₅ , 12°C. for 2 weeks	154.5 ± 1.2	..	72.7/17.1
V ₁₆ , 12°C. for 1 week	..	10	..
V ₁₇ , 2 weeks at 37°C. followed by 2 weeks at 12°C.	136.0 ± 1.3	..	62.5/19.1
V ₁₈ , 2 weeks at 12°C. followed by 2 weeks at 37°C.	..	12	..
V ₁₉ , 1 week at 37°C. followed by 1 week at 12°C.	144.0 ± 2.4	..	70.9/16.2
V ₂₀ , 1 week at 12°C. followed by 1 week at 37°C.	..	12	..
V ₂₁ , alternate day at 37°C. followed by 12°C. for 4 weeks	..	16	..
V ₂₂ , alternate day at 37°C. followed by 12°C. for 3 weeks	147.4 ± 1.2	..	68.8/14.3
V ₂₃ , alternate day at 37°C. followed by 12°C. for 2 weeks	152.6 ± 1.1	..	76.5/7.5
V ₂₄ , alternate day at 37°C. followed by 12°C. for 1 week	153.2 ± 1.4	..	79.5/10.6
V ₂₅ , 1 week at 12°C. followed by 4 days at 37°C. followed by 1 week at 12°C.	150.4 ± 0.85	..	75.8/9.5
V ₂₆ , 1 week at 12°C. followed by 4 days at 26°C. followed by 1 week at 12°C.	..	11	..
V ₂₇ , 1 week at 37°C. followed by 4 days at 12°C. followed by 1 week at 37°C.	..	14	..
V ₂₈ , 1 week at 26°C. followed by 4 days at 12°C. followed by 1 week at 26°C.	150.4 ± 0.85	..	75.8/9.5

EXPERIMENT 2

Effect of sowing time on the flowering of temperature treated seeds of Rupsail

The experiment was carried out in winter at the end of normal harvesting season of winter rice. It commenced on November 25 and seeds after treatments were sown on December 25, 1954, in earthen pots containing well manured garden

soil and exposed to natural day length (short days, varying from 10 hrs. 45 mins. and field temperature from 18°–24°C.).

The plants from different treatments were dissected on April 21, 1955 (i.e. after 116 days of sowing). The developmental stages of the shoot apex were denoted by scores (Table 3). The number of replicates for each treatment has been shown in brackets. Low temperature of the field induced slow growth rate of the plant and did not favour grain formation. The number of spikelets per ear was also reduced. Acceleration of flowering was not recorded, on the contrary delay was apparent at high temperature. By treatments with alternate temperatures for 3 weeks (S_{13} in Table 3) which caused earliness in the plants sown in normal rice season (Table 2, V_{22}) the score number was much advanced in comparison to other treatments.

TABLE 3

Effect of sowing time on floral initiation of Rupsail. Date of sowing on 25-12-1954, after different temperature treatments

Treatments	Score number
$S(c)$, control	21 (18)
S_1 , 37°C. for 4 weeks	4 (10)
S_2 , 37°C. for 3 weeks	19 (10)
S_3 , 37°C. for 1 week	17 (10)
S_4 , 26°C. for 4 weeks	Seeds not germinated
S_5 , 26°C. for 3 weeks	13 (9)
S_6 , 26°C. for 1 week	15 (10)
S_7 , 3°–5°C. for 4 weeks	Seeds not germinated
S_8 , 3°–5°C. for 3 weeks	17 (9)
S_9 , 3°–5°C. for 1 week	19 (13)
S_{10} , 2 weeks at 37°C. followed by 2 weeks at 3°–5°C.	3 (12)
S_{11} , 2 weeks at 3°–5°C. followed by 2 weeks at 37°C.	17 (11)
S_{12} , alternate day at 37°C. followed by 3°–5°C. for 4 weeks	15 (9)
S_{13} , alternate day at 37°C. followed by 3°–5°C. for 3 weeks	21 (18)

EXPERIMENT 3

Relation of short days to presowing temperature treatments of Rupsail. Date of sowing 15-6-1954 and short day application 7-8-1954

The significant effect of short days on winter varieties of rice is now a well established fact. The nature of this short day effect on vernalized plants was investigated in both winter and summer varieties (Sircar and Ghosh, 1954). Acceleration was noticed in the winter variety *Rupsail* by vernalization at 10°C. for 5 days followed by short days, whereas high temperature vernalization in the summer varieties was annulled by short day exposure. The present investigation was undertaken to ascertain further the effect of short photoperiods after treating the seeds with different temperatures for varying durations. After planting out the seedlings were grown in natural day length above 13 hours for 53 days. Subsequently short days of 8 hours for 4 weeks were applied. The plants were then kept in normal day length until ear emergence. The results (Table 4) indicate marked influence of short days on the acceleration of flowering but presowing low or high temperature treatment failed to induce further acceleration, on the contrary a delaying effect was noticed in some of the continuous and alternate temperature treatments. The fertility of the spikelets is reduced in the treatments inducing earliness.

TABLE 4

Presowing temperature treatments	Photoperiodic treatment after 53 days of natural day length	Number of days for ear emergence main shoot (average of 22-48 plants)	Spikelet numbers Fertile/Sterile
V_{c1} , soaked and sprouted at 26°C. for 24 hours	Natural daylight	150.5 \pm 1.7	100.2/16.2
V_{c2} , soaked and sprouted at 26°C. for 24 hours	8 hours' daylight for 4 weeks	97.5 \pm 0.17	65.2/29.0
V_1 , 37°C. for 4 weeks	"	99.6 \pm 0.57	42.2/31.0
V_3 , 37°C. for 2 weeks	"	99.5 \pm 0.84	49.5/43.4
V_5 , 30°-31°C. for 4 weeks	"	101.2 \pm 0.10	50.0/23.7
V_7 , 30°-31°C. for 2 weeks	"	98.2 \pm 0.11	57.0/39.4
V_9 , 25°-26°C. for 2 weeks	"	97.7 \pm 0.11	62.5/31.2
V_{11} , 25°-26°C. for 4 weeks	"	97.6 \pm 0.11	60.5/35.0
V_{13} , 12°C. for 4 weeks	"	101.1 \pm 0.74	38.5/37.6
V_{15} , 12°C. for 2 weeks	"	101.4 \pm 0.50	58.8/30.4
V_{17} , 2 weeks at 37°C. followed by 2 weeks at 12°C.	"	99.2 \pm 0.94	34.0/28.5
V_{18} , 2 weeks at 12°C. and 2 weeks at 37°C.	"	97.9 \pm 0.74	40.8/29.4
V_{21} , alternate days at 37°C. and 12°C. for 4 weeks	"	101.4 \pm 0.75	40.9/29.3

EXPERIMENT 4

Vernalization and devernialization of summer variety Dhairal

It has been reported previously (cf. Sircar, 1948) that presowing high temperature accelerates flowering in summer variety, while low temperature retards flowering. In order to analyse critically the relation of germination temperature of summer variety the present experiment was planned with treatments for continuous high or low temperature and alternate high and low temperatures for varying durations. The experiment was started on February 10 and seeds sown on April 11, 1955. The date of ear emergence of the main axis was recorded and presented in Table 5. The following facts are apparent. Effects of continuous high temperatures (30°-31°C.) were acceleration of flowering when the duration of the treatment was up to 3 weeks (P_{15} and P_{16}) but above this duration temperature has a delaying effect which increases with the duration of the treatment (P_{13} and P'_{14}). Similar effect was also noted by treatments at 37°C. (P_{18} and P_{19}). The low temperature (12°C.) for all durations, however, accelerates flowering which is statistically significant (P_{37} , P_{38} , P_{39} , P_{40}). Acceleration was also evident by treatments at 25°-26°C. for 4 weeks (P_{10}). On the other hand too low temperature (2°-4°C.) has a strong retarding effect on germination and left almost no replicates to assess significance on flowering (P_1 - P_4).

It is interesting to note that alternate temperatures have induced earlier flowering when high temperature is preceded or followed by low temperature (P_{35} , P_{36} , P_7). These effects are also statistically significant. Similar results were also noted in the case of the winter variety *Rupsail* (Table 2). A marked acceleration of flowering by low temperature treatment is accompanied by greater number of fertile spikelets, while in the rest of the treatment the spikelet number is less than that of the control and there is no definite correlation between the number of spikelets and mean date of ear emergence.

TABLE 5

Effect of presowing temperatures on floral initiation of Dhairal. Sowing date 11-4-1955

Temperature treatments	Mean days of ear emergence main shoot (18-25 plants)	Number of spikelets per ear Fertile/Sterile
P_c , control (soaked and sprouted at 26°C. for 24 hours) ..	111.1 \pm 1.45	85.5/11.5
P_1 , P_2 , P_4 , 2°-4°C. for 6, 4, 2 weeks	Not germinated	
P_3 , 2°-4°C. for 2 weeks (2 replicates) only	116.5	78.5/14.2
P_{37} , 12°C. for 6 weeks	105.1 \pm 1.44	67.7/16.1
P_{38} , 12°C. for 4 weeks	101.0 \pm 1.17	100.3/10.5
P_{39} , 12°C. for 3 weeks	101.8 \pm 1.21	106.2/6.0
P_{40} , 12°C. for 2 weeks	104.1 \pm 1.52	88.7/13.5
P_9 , 25°-26°C. for 6 weeks	117.1 \pm 1.20	72.5/8.5
P_{10} , 25°-26°C. for 4 weeks	105.1 \pm 1.77	80.2/10.5
P_{11} , 25°-26°C. for 3 weeks	113.5 \pm 0.88	75.6/11.2
P_{12} , 25°-26°C. for 2 weeks	108.8 \pm 1.16	87.6/7.5
P_{13} , 30°-31°C. for 6 weeks	123.5 \pm 0.5	82.5/14.5
P_{14} , 30°-31°C. for 4 weeks	120.3 \pm 1.4	69.3/12.0
P_{15} , 30°-31°C. for 3 weeks	109.4 \pm 1.32	83.3/12.4
P_{16} , 30°-31°C. for 2 weeks	104.2 \pm 1.32	88.2/14.3
P_{18} , 37°C. for 4 weeks	123.2 \pm 1.11	80.3/14.4
P_{19} , 37°C. for 3 weeks	119.1 \pm 1.03	76.7/13.3
P_{20} , 31°C. for 2 weeks	95.5 \pm 1.24	88.5/16.5
P_{21} , 4 weeks at 37°C. followed by 4 weeks at 2°-4°C. ..	122.1 \pm 0.88	72.9/15.7
P_{23} , 2 weeks at 37°C. followed by 2 weeks at 2°-4°C. ..	124.5	60.3/19.3
P_{24} , 3 weeks at 30°-31°C. followed by 3 weeks at 2°-4°C. ..	117.2 \pm 1.47	72 /12.3
P_{25} , 2 weeks at 30°-31°C. followed by 2 weeks at 2°-4°C. ..	118.3 \pm 1.32	70.7/11.4
P_{29} , 3 weeks at 2°-4°C. followed by 3 weeks at 30°-31°C. ..	116.5 \pm 0.5	80.3/11.5
P_{30} , 2 weeks at 2°-4°C. followed by 2 weeks at 30°-31°C. ..	113.3 \pm 0.69	85.3/12.5
P_{35} , 3 weeks at 37°C. followed by 3 weeks at 12°C. ..	104.7 \pm 1.73	60.1/10.2
P_{32} , 3 weeks at 37°C. followed by 3 weeks at 25°-26°C. ..	113.7 \pm 0.41	77.5/12.6
P_{36} , 3 weeks at 30°-31°C. followed by 3 weeks at 12°C. ..	103.8 \pm 1.6	85.2/13.2
P_{34} , 3 weeks at 30°-31°C. followed by 3 weeks at 25°-26°C. ..	109.3 \pm 1.9	80.2/13.0
P_6 , 3 weeks at 25°-26°C. followed by 3 weeks at 37°C. ..	120.4 \pm 1.32	70.2/17.5
P_8 , 3 weeks at 25°-26°C. followed by 3 weeks at 30°-31°C. ..	115.2 \pm 0.63	67.2/19.3
P_7 , 3 weeks at 12°C. followed by 3 weeks at 37°C. ..	99 \pm 1.38	107.2/13.3

DISCUSSION

This paper provides evidence for a detail survey of temperatures of germination on flowering of winter and summer varieties of rice. Previous results with rice (cf. Sircar, 1948) indicate that hastening of flowering by varying temperature treatments is not so effective as that found in the temperate crops like rye. The winter variety of rice which was considered to be a temperature neutral plant showed a significant acceleration of flowering under exposure to alternate high and low temperatures of germination while continuous high or low temperature proved to be ineffective.

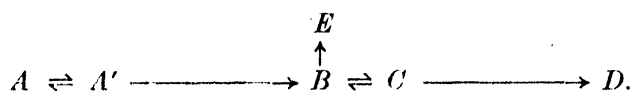
Rice is a tropical crop; it grows generally at a temperature about 27°C. to 33°C. under field conditions. The temperature near freezing point seems to have a lethal effect on rice embryo as most of the embryos were killed at 2°-4°C. and thus the percentage of germination was severely affected. This lethal effect of low temperature was more evident in the case of summer variety. Germination rate was also significantly reduced when such low temperature either preceded or followed high temperature treatment. At slightly higher level of temperature (12°C.) an acceleration of flowering of the summer variety was noticed in all the treatments, the effect increased with the increase of duration (P_{40} , P_{39} , P_{38}). At still higher temperatures 25°-26°C. and 30°-31°C. acceleration of flowering was also found but the

effect was not so great as that in the case of 12°C. and generally decreased with increase of duration; thus acceleration at 30°-31°C. was found only up to 2 weeks' duration above which the effect of temperature did not show hastening. With still prolonged duration for 4-6 weeks a significant retardation was evident (P_{14} and P_{15}).

These results, however, do not agree with the previous findings of the vernalization temperature of rice which was found to be higher than the normal temperature (cf. Sircar, 1948). On the contrary, a generalization of the effect on the vernalization temperature between temperate and tropical crops can be shown in the following way: that the optimal temperature for vernalization in both temperate and tropical crops lies well below the normal temperature and the effect is extended and gradually slowed down up to the level of normal field temperature of respective regions (12°-15°C. for temperate crop and 30°-31°C. for tropical crop).

In the light of the results obtained in the experiments reported above the scheme put forward by Purvis and Gregory (1952) requires consideration.

Their revised scheme is represented below:—



' A represents the precursor from which a specific substance B is produced by a reaction at low temperature. The reaction $A \longrightarrow B$ consists of at least two stages, the first $A \rightleftharpoons A'$ is reversible by high temperature, so that devernalization and reveralization are possible. The second stage $A' \longrightarrow B$, however, can proceed either at normal or low temperatures. The substance B is evidently thermostable and C is produced by a reversible reaction proceeding forward in darkness (short day induction) and reversed in light. The reaction $C \longrightarrow D$ proceeds only in light. C and D may be regarded as substances responsible for flower initiation and development and these reactions occur in the terminal meristem. The substance B alternately may be converted into the substance E which favours leaf production.'

Friend and Gregory (1953) have assumed further that the postulated ' B ' the end-product of the vernalization reaction increases auto-catalytically and that the rate of the reaction increases with temperature. The precursor A is present in the winter variety of rye which is practically temperature neutral variety; the thermostable substance B is assumed to be present there.

In the present study it is suggested that the precursor A may be present in the summer variety of rice which is vernalized by low temperature 12°C. (Table 5, P_{37} , P_{38} , P_{39} , P_{40}) and devernalized by supra-normal temperature (P_{29} , P_{30}). But in the case of winter rice the precursor A might have already been converted into substance A' in the mature grain and in the remaining part of the reaction B can only produce D by short day induction and in no case the low temperature has any influence on hastening of flowering (Table 2, V_{14} , V_{15}). The delaying effect of high temperature on winter variety of rice (Table 2, V_6 , V_7 , V_{11}) may be argued as 'devernalization' reaction as the reaction $A \rightleftharpoons A'$ is reversible. Again by application of alternate high and low temperature such delaying effect of high temperature is annulled showing the reaction $A \rightleftharpoons A'$ may be proceeded forward (Table 2, V_{17} , V_{19} , V_{22} , V_{23} , V_{24}). It is assumed that B is produced auto-catalytically with temperature and thus the quantity B may be increased which intensifies the vernalization reaction. Such reaction may possibly be the causal factor for 14 days' acceleration of flowering in winter variety of rice under exposure to alternate high and low temperature.

Delaying effect of high temperature on winter variety is also found in the plants either sowing later or treated photoperiodically (Table 3, S_1 , S_2 , S_3 , S_5 and Table 4, V_5) and such effect of high temperature is annulled under exposure to subsequent low temperature verifying the assumption presented in this paper.

ACKNOWLEDGEMENTS

We desire to express our thanks to Dr. I. Banerjee, Head of the Department of Botany, for facilities offered in this investigation and to Dr. T. M. Das for valuable suggestions and unreserved help.

SUMMARY

During germination seeds of winter and summer varieties of rice were treated with different degrees of continuous and alternate ranges of temperatures for varying duration.

In winter variety high and low temperatures show retardation of flowering while acceleration was recorded at alternate exposures of high and low temperatures. Similar results were obtained with late sowing in winter. The effect of short day induction, however, did not intensify the vernalizing reaction.

In summer variety an acceleration of flowering was noticed at subnormal temperature. The optimal degree for vernalization was 12°C. the effect of which extended and gradually slowed down up to the level of 30°-31°C. the normal field temperature of the crop. Above normal temperature (i.e. at 37°C.) a devernalization effect was found which increased with duration. Such retarding effect of high temperature was annulled under subsequent exposure to low temperature. The results have been discussed in the light of the scheme put forward by Purvis and Gregory and a generalization of the effect of temperature for vernalization of temperate and tropical crops is presumed.

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STUDIES ON CYTOCHEMISTRY OF HORMONE ACTION

PART XVI. FURTHER EVIDENCE OF THE ENHANCEMENT OF ANDROGENIC ACTION OF TESTOSTERONE PROPIONATE BY PROGESTERONE

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It seems satisfactorily established that progesterone possesses appreciable androgenic activity as tested by histophysiologic and metabolic responses of the male accessory genital organs to this hormone (for earlier references see Kar, 1949; Kar and De, 1953; and Price *et al.*, 1955). The possibility that progesterone can add to the androgenic action of testosterone was, however, originally suggested in the experiments of Morgan (1946) who showed that progesterone synergized the restoring effect of testosterone on the accessory genital organs of spayed female opossums. Subsequently, Kar and De (1953) were able to demonstrate that progesterone enhanced the androgenic effect of testosterone propionate when injected simultaneously in castrated male rats. The recent findings of Price *et al.* (1955) also tend to subscribe towards a similar physiological relationship between progesterone and androgens.

In the present paper, an attempt has been made to provide further evidence of the enhancement of androgenic effect of testosterone propionate by progesterone.

The literature on metabolic activities of the male accessory genital organs is replete with references on a positive correlation between androgenic activity and the concentration of alkaline phosphatase (Roberts and Szego, 1953). Because of the consistency of such a correlation, it has even been suggested that fluctuations in alkaline phosphatase activity in the male accessory genital organs can be used as a sensitive chemical assay method for small amounts of androgen (McCullagh and Schaffenburg, 1954). In view of these, in the present study, the alterations in alkaline phosphatase concentration in the seminal vesicles of castrated rats have been utilized as the criterion for the evaluation of androgenic effects of progesterone whether given alone or in combination with testosterone propionate.

EXPERIMENTAL PROCEDURE

Thirty adult albino rats of the Institute colony, weighing on an average 170 gm., were used in this study, out of which twenty-four were castrated. The unoperated rats and a group of six castrates served as the controls. The castrates were assigned in three groups of six animals each for receiving hormone treatments. The latter was initiated on the 31st day following castration and before the commencement of the injections, the seminal vesicles of all the castrates were examined by laparotomy in order to ensure that these had adequately regressed. All of the animals were maintained under uniform laboratory conditions throughout the experimental period.

Progesterone and testosterone propionate were injected by the intramuscular route in daily doses of 2.5 mg. (in 0.1 ml. of sterile olive oil) of each hormone for

10 days. This dosage was used for the groups which received the two hormones separately. For the group which was subjected to combined treatment 2.5 mg. of each hormone per day was injected simultaneously so that a total of 25 mg. of progesterone plus an equal amount of testosterone propionate were given per animal. The control castrate and the unoperated rats (Table I) received 0.1 ml. of sterile olive oil alone daily for the same period. The dosage used in the present study was essentially similar to that used previously (Kar and De, 1953).

The animals were sacrificed 24 hours after the final treatments. The seminal vesicles were carefully dissected out and fixed immediately in chilled 80 per cent ethyl alcohol. The serial paraffine sections of the organ (6 micra thick) were processed according to the technique of Gomori (1941) as laid down by Glick (1949) for the demonstration of alkaline phosphatase.

RESULTS

Controls.—The pattern of distribution and concentration of alkaline phosphatase in the seminal vesicles of normal rats agreed with those of the previous workers (Bern, 1949; Melampy and Cavazos, 1953). The epithelium gave an entirely negative reaction for the enzyme and the lamina propria was only faintly positive. The latter was true for both the connective tissue and the vascular elements of the lamina propria. The muscularis gave a patchy and diffuse reaction (Pl. XVIII, Fig. 1).

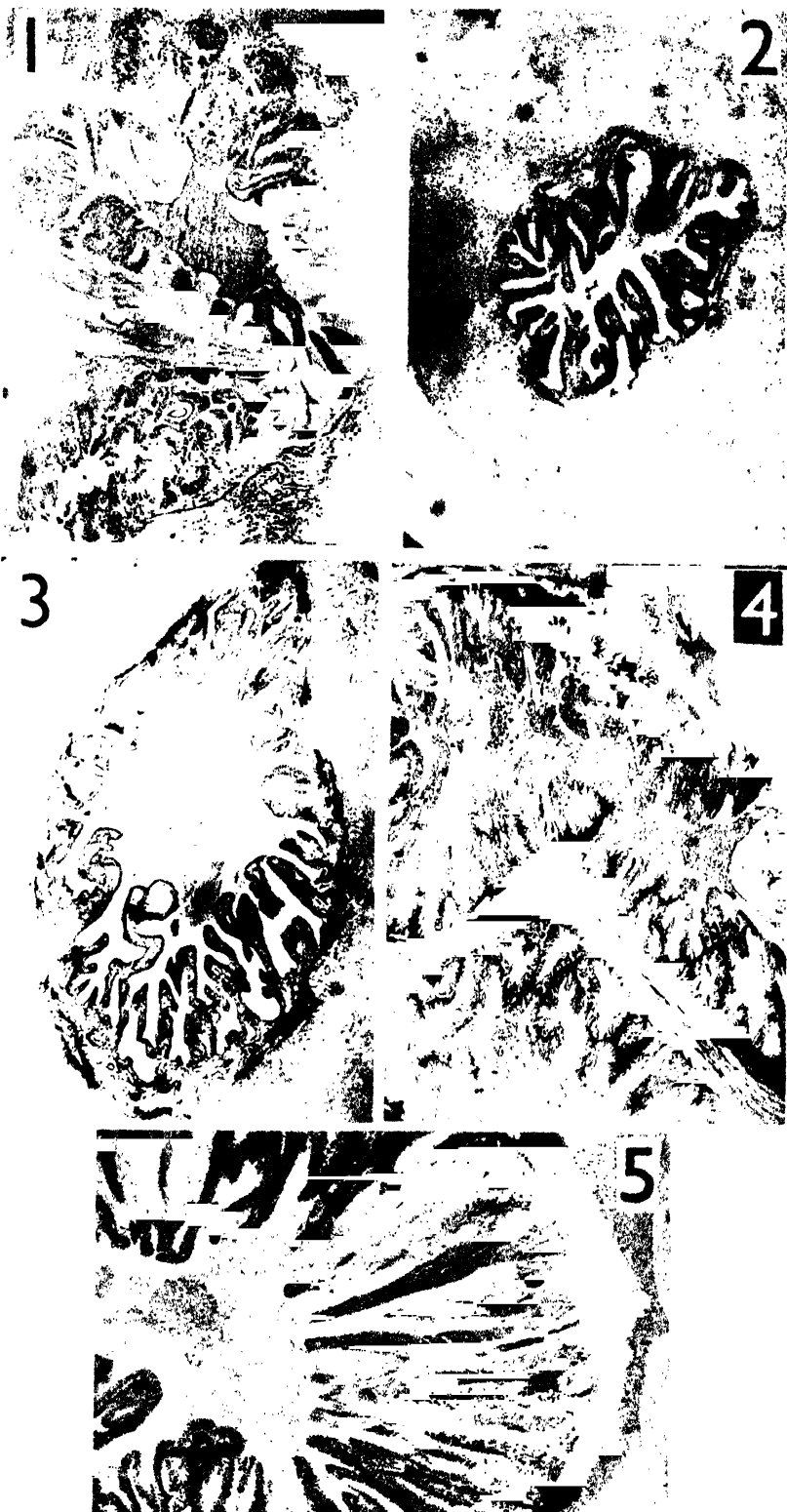
TABLE I

The distribution and concentration of alkaline phosphatase in the seminal vesicles of normal and experimental rats

	Controls	Castrates	Castrates + proges- terone	Castrates + testos- terone propionate	Castrate + proges- terone + testos- terone propionate
<i>Epithelium</i>					
Nucleus	0	0	0	0	0
Cytoplasm (including secretion gran- ules)	0	0	0	0	0
<i>Lamina propria</i>					
Connective tissue ..	+ p	+ p	+ + p	+ + +	+ + + +
Blood vessels ..		0	+	+ +	+ + +
<i>Muscularis</i>					
Smooth muscle ..	+ p	0	0	0	+ + +
Blood vessels ..	0	0	0	+ + +	+ + +

Evaluation.—

- + + + + = Intense phosphatase activity.
- + + + = Strong phosphatase activity.
- + = Weak activity.
- + = Faint reactions.
- + p = Faint and patchy reactions.
- 0 = Negative reaction.



(*castrates*.—The epithelium was negative for the phosphatase as in the normal controls. The other components of the organ were also devoid of phosphatase activity except for a faint reaction in the lamina propria (Pl. XVIII, Fig. 2 and Table I).

(*castrates + progesterone*.—The epithelium continued to give a negative reaction for phosphatase activity but the connective tissue of lamina propria was weakly positive (Pl. XVIII, Fig. 3). The blood vessels of the lamina propria stained faintly for the enzyme. The overall picture, however, was slight stimulation of phosphatase activity as compared to that of the untreated castrates.

(*castrates + testosterone propionate*.—No reaction for phosphatase activity was seen in the epithelium. The connective tissue of the lamina propria showed strong reactions but the vascular elements were only weakly positive. In the muscularis only the endothelium of the blood vessels gave strong reactions for the enzyme (Pl. XVIII, Fig. 4). The overall picture was considerable stimulation of phosphatase activity in sharp contrast to the untreated castrates and the normal controls (Pl. XVIII, Fig. 4).

(*Castrates + progesterone + testosterone propionate*.—The epithelium was negative for phosphatase activity. The connective tissue of the lamina propria gave intense reactions for the enzyme and the blood vessels were also strongly positive (Pl. XVIII, Fig. 5). In the muscularis, both the fibres and the blood vessels showed strong concentrations of the enzyme. The extent of overall increase in alkaline phosphatase activity was greater than that of the androgen recipients.

DISCUSSION

The results of the present study tend to bear out and extend the findings of Kar and De (1953) regarding the enhancement of androgenic action of testosterone propionate by progesterone. It is interesting that, at the dosage level used in this study, the weight and gross histological responses of the seminal vesicles of castrates to progesterone are negligible (Kar and De, 1953). This is to be expected as according to the recent estimation of Price *et al.* (1955) a massive dose of progesterone, several times greater than that used by us (*loc. cit.*), is necessary to reveal its androgenicity in the castrates when given alone. Nevertheless, a careful perusal of the phosphatase picture of the seminal vesicles of rats treated with progesterone (Pl. XVIII, Fig. 3) will indicate a slight but definite stimulation of phosphatase activity even at this otherwise low dosage. This not only emphasizes the sensitivity of the enzymic response in the detection of trace amounts of androgenicity but also leads to the possibility that in course of recovery of an accessory male genital tissue from the effects of chronic testicular insufficiency some metabolic features of the tissue is restored earlier than its gross morphology. The significant histochemical findings of Melampy and Cavazos (1953) can be brought to bear upon such a viewpoint. These workers reported that the activity of alkaline phosphatase in the seminal vesicles of castrated rats is restored to supernormalcy on the fifth day of androgen therapy but thereafter a relative decline in enzyme activity from the supernormal level is clearly noticeable. According to the present hypothesis, this may be due to a sequential restoration of normal phosphatase activity at a very early stage of androgen therapy, the subsequent attainment of a supernormal level with time, and final interference with the kinetics of the enzyme by sustained hormonal stimulation. To what extent such a picture parallels the physiology of the tissue concerned will be an interesting topic for research.

A final consideration of the above facts and possibilities, therefore, leads to the inference that progesterone can enhance the androgenic effect of testosterone propionate. This is indicated by changes in weight and gross histology of the seminal vesicles of castrated rats as reported earlier (*loc. cit.*) as also by the responses of a specific enzyme, the alkaline phosphatase, which is known to have a positive correlation with androgenicity.

SUMMARY

1. Castration causes disappearance of alkaline phosphatase activity from the seminal vesicles of the rat.
2. Progesterone slightly stimulates phosphatase activity in the seminal vesicles of similar animals but testosterone causes a strong mobilization of the enzyme in this tissue.
3. Combined therapy with progesterone and testosterone propionate is associated with a greater stimulation of phosphatase activity than is noticed with the androgen alone.
4. Previous findings on the enhancement of androgenic effect of testosterone propionate by progesterone is confirmed.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Dr. B. Mukerji for his interest in this study. Thanks are due to Sri S. Banerji for the photomicrographs.

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EXPLANATION OF PLATE XVIII

(All figures are photomicrographs and are of equal magnification)

- FIG. 1. Transverse section through the seminal vesicles of a control rat. Note the patchy distribution of alkaline phosphatase in the lamina propria.
- .. 2. Transverse section through the seminal vesicles of a castrated rat. Note the patchy and diffuse reactions for the enzyme in the lamina propria.
- .. 3. Transverse section through the seminal vesicles of a castrated rat treated with progesterone. Note weakly positive reactions in the lamina propria.
- .. 4. Transverse section through the seminal vesicles of a castrated rat treated with testosterone propionate. Note the hypertrophy of the organ and stimulation of phosphatase activity.
- .. 5. Transverse section through the seminal vesicles of a castrated rat treated with testosterone propionate + progesterone. Note the conspicuous foldings of the mucosa and pronounced stimulation of phosphatase activity.

THE INCIDENCE AND HEREDITY OF HAEMOPHILIA IN INDIA

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INTRODUCTION

There has been, so far as the present writer's knowledge goes, only one study of haemophilia in India (Ghosh, 1942) in which one Bengali family from Calcutta was described. Further studies on this family have been presented here. The other study relating to a Muslim family of Indian stock was made in 1859 by Heymann (*cit.* Gates, 1946) from Palembang, Sumatra.

Apart from the clinical symptoms peculiar to haemophilia, its sex-linked recessive behaviour has been accepted to be one of its diagnostic features. This hereditary behaviour has been emphasized so much that in many cases, in spite of the typical clinical findings of the disease, haemophilia has been ruled out because the typical sex-linked heredity is not seen in the family. The latest case on record is from Australia (Fantl and Margolis, 1955). It is a genetic puzzle since haemophilia in both the mother and the son has been confirmed through the latest clinical examination of the blood. Gates (1946) in a detailed genetic study of this disease has shown that haemophilia, like many other diseases and abnormalities, may not always follow the sex-linked recessive type of inheritance and that y -linked, simple dominant or recessive forms of inheritance may also occur. According to Gates the inheritance of haemophilia may also vary within the same sib due to the cross-over of the gene from x -chromosome to the y -chromosome or vice versa. This fact should not be lost sight of in giving weight to the hereditary behaviour as a diagnostic feature of haemophilia.

Since 1947, a number of cases with haemorrhagic diathesis has been called parahaemophilia (1952). It differs from the classical haemophilia in having a prolonged prothrombin time, which is normal in the case of haemophilia. Like haemophilia it does not affect the joints. It is not sex-linked in inheritance since both the sexes are known to be affected and does not always show a family history. It is associated with the deficiency of the accelerator globulin and should be regarded as a separate syndrome.

Recent discovery of 'Christmas disease' or PTC deficiency (Biggs *et al.*, 1952) shows all the genetic and clinical features of haemophilia but differs from the latter in the presence of anti-haemophiliac globulin, and in the deficiency of some other factor, essential for the formation of thromboplastin. This has led to the assumption of an allelomorphous variant of the gene responsible for haemophilia. Even a series of allelic genes, which determine the plasma level of the anti-haemophiliac factor, has been postulated in the case of mild haemophiliacs. Improved laboratory methods of diagnosis have been established by Biggs and Macfarlane (1953) and Fantl (1954) and these should be followed in this country as well. Fantl proposes that classical haemophilia be called alpha-prothromboplastin deficiency while Christmas disease or PTC deficiency that of beta-prothromboplastin deficiency.

Haemophilia in homozygous females is now an established fact (Merskey, 1951) although Gates (1946) made a case of it through Treves' pedigree of 1886 and its later studies by Bulloch and Fildes in 1911 and Handley and Nussbrecher (1935)

and recently by Merskey (1951). Lloyd (1925) showed that incomplete dominance of the normal condition may also show female bleeders.

It is peculiar, however, that Andreassen (1943), who carried out a complete survey of haemophilia in Denmark and published 63 pedigrees of this disease, has neither found any deviation from the classical form of the disease nor in its sex-linked inheritance. He has not found any genuine haemophilia in the females, though he has noted a tendency to haemorrhage in several heterozygotes, whose blood showed a slight protraction of the coagulation time. This, however, is not confirmed by Merskey and Macfarlane (1951).

Method

In order to find out the incidence of haemophilia in India, a questionnaire was first of all sent to 40 hospitals throughout the country. After the replies were obtained, attempts were made to enquire into the genealogy of each case. For this purpose a tour to Bombay, Nagpur and Ahmedabad, was undertaken and family histories from the above three places were collected. For other cases, enquiries were made through correspondence. The Bengal pedigree was collected after personal interviews from various sources.

The Data

Out of 40 questionnaires sent to various hospitals only 21 replies were received. The number of haemophiliacs reported therefrom is given in Table 1.

TABLE 1
Number of Haemophiliacs reported from Hospitals.

Serial No.	Hospital	City	Period	No.
1	Sarojini Naidu	Agra	1951-54	0
2	Civil	Ahmedabad	1953-54	1
3	Victoria Jubilee	Amritsar	1951-54	5
4	S. S. General	Baroda	1954	0
5	S. G. S. Medical College	Bombay	1945-54	13
6	Dental College	Calcutta	1954	0
7	Medical College	"	1952-54	0
8	N. R. Sircar Medical College	"	1954	0
9	S. C. B. Medical College	Cuttack	1954	0
10	Assam Medical College	Dibrugarh	1953-54	1
11	G. R. Medical College	Gwalior	1949-54	0
12	M. G. M. Medical College	Indore	1950-54	0
13	S. M. S. Medical College	Jaipur	1954	0
14	G. M. & Associated	Lucknow	1951-53	0
15	Medical College	Madras	1950-54	3
16	Stanley Medical College	"	1952-54	0
17	University Medical College	Mysore	1934-54	0
18	Medical College	Nagpur	1954	1
19	Irwin	New Delhi	1954	1
20	Christian Medical College	Vellore	Past several years.	2
21	Andhra Medical College	Vishakapatnam	1944-54	0
				27

It will be seen from Table 1 that out of 21 hospitals, haemophilia has been reported from Ahmedabad, Amritsar, Bombay, Dibrugarh, Madras, Nagpur, New

Delhi and Vellore. The rest has not treated any case of haemophilia. The longest record of absence of any case is from Mysore during the period 1934-54, followed by that of Vishakapatnam during 1944-54. Gwalior and Indore had no cases during the past 5 and 4 years respectively.

The highest incidence appears to be from Bombay during the last 9 years (1945-54). It is likely that there are more cases since only one, out of the seven Bombay hospitals approached, has replied to our query. The next incidence with 5 cases is from Amritsar during the years 1951-54. Amritsar appears to have a higher incidence as the pedigree data will reveal afterwards. Madras Medical College hospital has reported 3 cases during 1950-54 while the Stanley Medical College hospital, Madras, has not treated any case of haemophilia during 1952-54. The Christian Medical College hospital, Vellore, has treated two cases during the past several years but none of them could be traced according to the addresses given by the hospital. Each of the other four hospitals, namely Civil hospital, Ahmedabad, Assam Medical College hospital, Dibrugarh, Medical College hospital, Nagpur, and the Irwin hospital, New Delhi, has treated one case during the year 1953-54. Their previous records could not be obtained.

Due to the highest incidence of haemophilia from Bombay, detailed enquiries of the 13 cases reported by the K. E. M. hospital, Bombay, were undertaken during January 1955.

(a) *Haemophilia cases from Bombay*

Case No. 1. M. R., male, aged 19 years, was admitted on 12th December, 1945, with swollen knee joints and bleeding gums. Past history revealed that the patient bleeds for a long time whenever he is hurt. Family history revealed no such condition in any member of the family. The patient has 12 normal brothers. Clinical examination of the patient's blood showed: bleeding time— $2\frac{1}{2}$ min.; coagulation time—30 min.; platelets—217,600/ccm.; blood group—O.

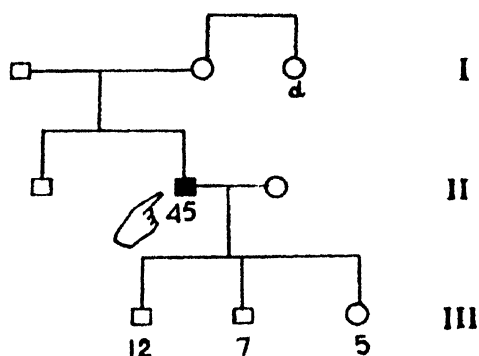
Personal enquiries revealed that the above patient is a resident of the village of Libola, Panchmahals. He obtained admission through a cook of the hospital, who informed the writer that the patient was seen alive about 6 months ago. The patient was married but the cook could not inform anything of his issues; neither could he say anything regarding the disease in the heredity of the patient. The patient is a farmer by occupation.

Case No. 2. K. M. M., male, aged 30 years, was admitted on 5th February, 1948, with bleeding gums and previous history of bleeding from trivial cuts from childhood. The past history also revealed that at the age of 6 he was operated on the forehead when the bleeding was controlled with great difficulty. Every 2-3 months he bleeds and about 6 years ago he had a bleeding which lasted 21 days. The clinical details of the blood are: bleeding time—1 min.; coagulation time—22 min.; platelets—94,000/ccm.

On interview the patient (Ped. 1, 11₂) was found to be quite healthy. He had a defective leg, probably a case of dislocation of the thigh, which the patient said has nothing to do with haemophilia. He was then having homoeopathic medicines, which were doing him good. He does not remember to have known anybody suffering from this disease among his relatives, specially on the mother's side. He is a businessman by occupation and is married, with three minor children. The pedigree is given below.

Case No. 3. G. E., male, aged 17 years, was admitted on 10th June, 1950, with both the knee joints swollen and the kidney affected. He had this recurrent swelling of the knee joints since the last 10 years apart from bleeding from trivial injuries. About a year ago he had haematuria which lasted for about 15 days. The clinical details of the blood are: bleeding time—2 min.; coagulation time—19 min.; platelet count—195,200/ccm.; prothrombin time—20 sec.

PEDIGREE 1*



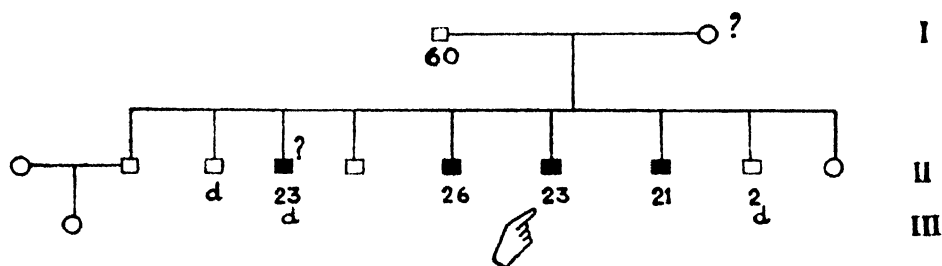
This patient could not be traced at Bombay. The hospital records also do not show any details of the family history.

Case No. 4. N. N., male, aged 7 years, was admitted at the hospital on January, 1951, with recurrent attack of bleeding from nose since 1 year of age. Clinical details of the blood are: bleeding time— $4\frac{1}{2}$ min.; coagulation time—3 min.; platelets—198,240/cmm.

This patient could not be traced at Bombay. The hospital records, however, mention two brothers of the patient having died of similar complaints at the ages of 8 and 10 respectively.

Cases Nos. 5 and 7. These two cases, though separately shown by the hospital, have been combined together because the author's investigations showed that they are related as brothers (Ped. 2, II 6 and 8). C. H. (Ped. 2, II 8), male, aged 17 years, was admitted on 17th May, 1951, with the symptom of bleeding from rectum. Clinical picture of the blood was: bleeding time—1 min. 50 sec.; coagulation time—13 $\frac{1}{2}$ min.; platelets—281,600/cmm.; prothrombin time—45 sec.; blood group—

PEDIGREE 2



* *Key to the Symbols used.*

Normal male	□
„ female	○
Haemophiliac	■ ●
Carrier	⊙
Months	m
Dead	d
Propositus	☞
Twin	Λ
Figures indicate age	
No ch.	No children

M. H. S. (Ped. 2, II 6), male, aged 19 years, was admitted on 22nd August, 1951, with the history of repeated swelling of both the knees. No external haemorrhages were noticed. Nothing is known of the clinical details of the blood except that he belonged to blood group B and that the patient was discharged on 24th August, 1951, after a blood transfusion.

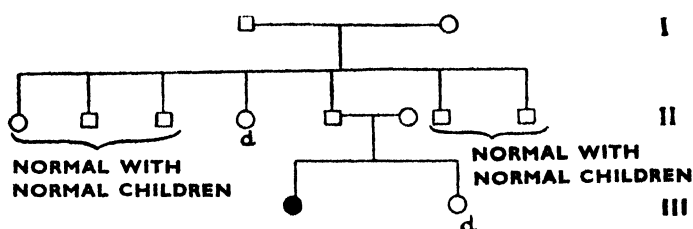
Personal enquiries into the family revealed that a brother (II 4) also died in 1948 from the same disease at about the age of 23, while there is another haemophilic brother (II 7) who is still suffering. All the brothers have affected knee and elbow joints and the former are said to be more troublesome than the latter. External haemorrhages are not apparent and all the three brothers are now grown up and conscious to avoid injuries. The mother (I 2) is affected with very frequent petechial haemorrhages but none of her relatives show any symptoms of the disease. The family is a refugee one from Karachi and the father of the family, an old man of about 60 years, informed the writer that this disease is the only case of its kind among the Sindhi Hindus. His son (II 8) was first treated in a Karachi hospital and because of the rare nature of the disease he was demonstrated before a medical conference comprising the local medical men. He was extremely emphatic on the point that his was a small community and he has never heard of this disease in any of its members.

Case No. 6. S. T., male, aged 5 years, was admitted on 13th June, 1951, due to bleeding after an operation for phymosis. His blood picture was: bleeding time—2 min. 10 sec.; coagulation time—7 min. 40 sec.; blood group—O.

On interview the boy was found to be quite normal. The mother and the father's sister of the boy informed the writer that neither the boy nor any member of their families has ever suffered from any kind of haemorrhage. The boy is the eldest issue of the family and is followed by two brothers, all in normal health.

Case No. 8. S. K. (Ped. 3, III 1), female, aged 1½ years, was admitted on 6th November, 1955, with bleeding gums. She had a previous history of bleeding from an incisor abscess and from trivial injuries, which gave no response to coagulants. Her blood picture was: bleeding time—4 min. 53 sec.; coagulation time—3 min. 5 sec.; platelets—268,000/cmm.; blood group—A.

PEDIGREE 3



On interview the child was seen to be quite healthy. Her father's eldest sister (II 1) informed the present writer that she last bled from the nose and gums about 6 months ago and her bleeding tendency was noticeable from a very early age. Each time she bleeds profusely; the joints are not affected. The severity of bleeding has caused the child to be treated in three different hospitals, one after the other, just with the idea of improved treatment. There is no affected individual in the family and the father's eldest sister (II 1), an old woman of about 65 years, was emphatic about it.

Case No. 9. M. S., male, aged 5 years, was admitted on 10th June, 1952, with the symptom of spontaneous bluish indurated swelling on any part of the body. He showed this symptom since 1 year of age. His blood showed: bleeding time—1 min. 25 sec.; coagulation time—not clotted at the end of one hour; platelets—206,720/cmm.

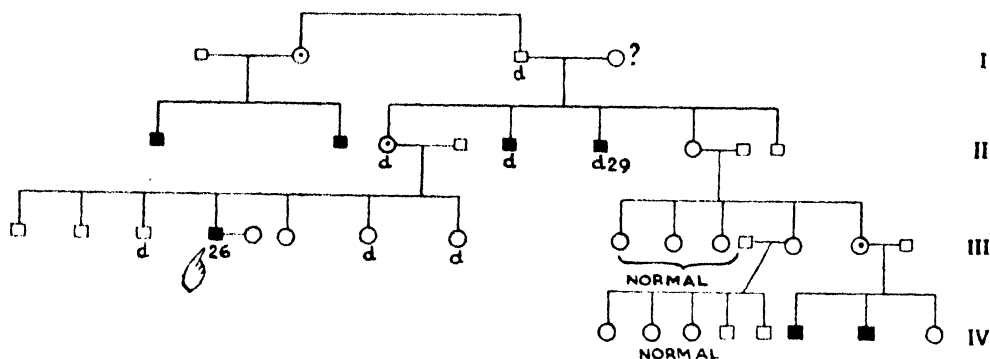
On interview at the address given by the hospital, it was found that the family had gone out of Bombay about 6 months ago, when the child was living. A friend

of the family informed that the boy was treated in another hospital after his discharge from the K. E. M. hospital.

Case No. 10. H. G., male, aged 24 years, European (?) (Ped. 4, III 4), was admitted on the 31st October, 1952, with swollen knee joints. He had it about 10 days ago and gave a history of bleeding from trivial injuries since birth and had haemophysis about 1 year ago. His blood showed: bleeding time—2 min. 7 sec.; coagulation time—6 min. 20 sec.; platelets—232,200/ccm.; blood group—A.

This patient could not be contacted although his church became interested in him. The hospital records, however, contained a genealogy, lacking in many details, which is given below:

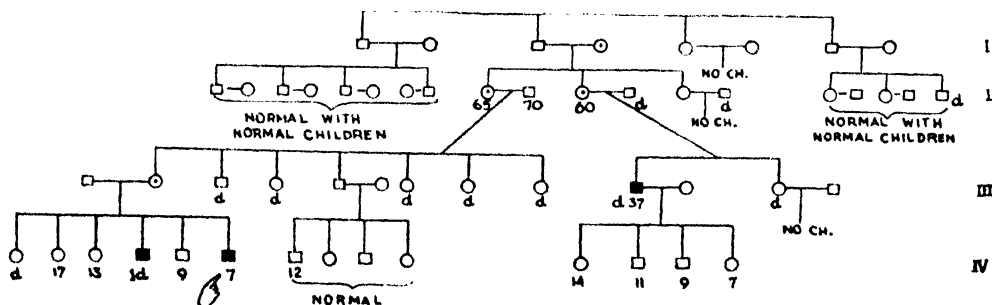
PEDIGREE 4



It will be seen from the pedigree that haemophilia is present in two brothers of the mother and they died of this disease probably. II 5 used to bleed very often from cuts and bruises and died during lifting a weight, while II 6 died of bleeding from an abscess, operated upon the neck at the age of 29. The father (I 3) is known to be normal but has a carrier sister, while the behaviour of the disease in the children of I 3 indicates that the mother was also a carrier. There appears to have been some amount of inbreeding in the family. No first hand information could be gathered on this family and nothing definite can therefore be said.

Case No. II. K. Y. J. (Ped. 5, IV 6), male, aged 4 years, was admitted on the 15th December, 1952, with affected skin. His father gave histories of echymoses since birth and one of his elder brothers (IV 4) died of prolonged haemorrhage after a trivial injury on the foot at 1 year of age. His blood showed: bleeding time—1 min. 45 sec.; coagulation time—27 min.; prothrombin time—30 sec.; platelets—345,600/cm.

PEDIGREE 5

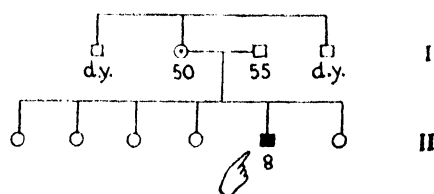


On interview the boy (IV 6) was found to be quite active and healthy. He bled from his gums three days ago and echymosis appears quite frequently. The

maternal uncle of the boy (III 3) died at the age of 8 from an accidental fall while the other uncle (III 5) is normal and is employed out of Bombay in a transport service. All the maternal aunts died young and unmarried. It will be seen from the pedigree that another maternal uncle (III 10) of the boy was also a haemophilic and the hospital papers relating to the boy also contain some references of III 10. This patient was also treated at the K. E. M. hospital but his papers could not be traced. The present writer was informed by a distant relative, an old employee of the hospital, of the above patient (III 10) that the latter was admitted into the hospital by him while he was about 12 years old. He was constantly suffering from echymosis and haemorrhages and died in 1954 at about the age of 37 leaving 2 sons and 2 daughters. Unfortunately, both II 9 and II 11 are living in two different places outside Bombay city and could not be contacted. Their third sister (II 13) is a widow without any children. The probability of I 4 being a carrier is however great. The family is a native of the state of Bombay.

Case No. 12. P. D. (Ped. 6, II 5), male, aged 5½ years, was admitted on 17th March, 1953, with affected knee joints. He had this swelling several times during the past 1 year and before coming to the hospital he was bleeding from the gums for about 5 days. Previously, he was treated in another hospital in the city. His blood showed: bleeding time—1 min. 55 sec.; coagulation time—more than 1 hour; platelets—246,400/ccm.; prothrombin time—noted as 'quick'; blood group—A.

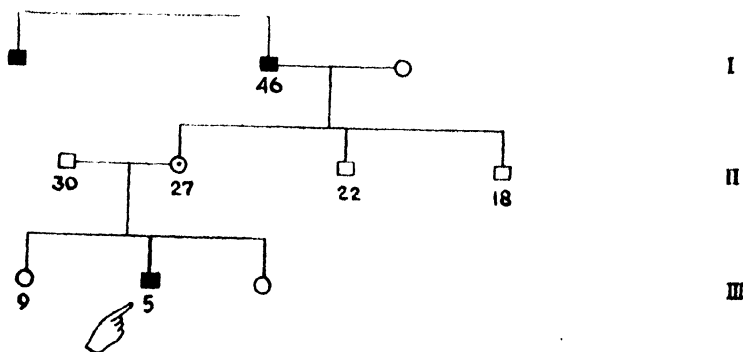
PEDIGREE 6



On interview the boy was seen suffering from swollen knees and was bleeding from the gums. The mother of the boy could not say the cause of death of her two brothers. The family is a native of the state of Bombay.

Case No. 13. S. G. (Ped. 7, III 2), male, aged 4 years, was admitted on the 23rd April, 1954, with bleeding gums and teeth. He had repeated attacks of bleeding since the last 1 year. His blood showed: bleeding time—3 min.; coagulation time—6 min.; platelets—350,000/ccm., blood group—A.

PEDIGREE 7



On interview the boy was found to be quite healthy. The father of the boy also said that he has been keeping well for some time past. The pedigree shows bot

the mother's father and his brother as haemophiliacs. The union of a haemophilic male and a normal woman results in normal sons and carrier daughters. In the present case this is seen very well. The only daughter of the union has been a carrier, the mother of the child in this case. The family is a native of Ramnad, S. India.

(b) *Cases from Ahmedabad*

Case No. 14. A. C., male, aged 24 years, was admitted at the Civil hospital, Ahmedabad, on 21st January, 1953, with pain and an intra-abdominal lump in the right iliac fossa during the last 4 days. His past history revealed an injury at the age of 6 years on the forehead from which he bled continuously for 15 days. Then at the age of 12 he injured his finger which also bled for 10-12 days. His joints were affected. His blood showed: bleeding time—2 min.; coagulation time—23 min.; platelets—206,000/cem.; prothrombin time—18 sec.; blood group—A. The patient was not colour blind.

The patient refused to give any details of his family history. His parents are normal and healthy and are living in a distant village. His eldest brother got the similar complaint of pain with lump in the abdomen and died on the 3rd day of attack at the age of 28. He had also similar bleeding tendency. The patient has two more younger brothers, who are said to be normal and healthy. There are no sisters. The patient is married and is a native of Gujarat.

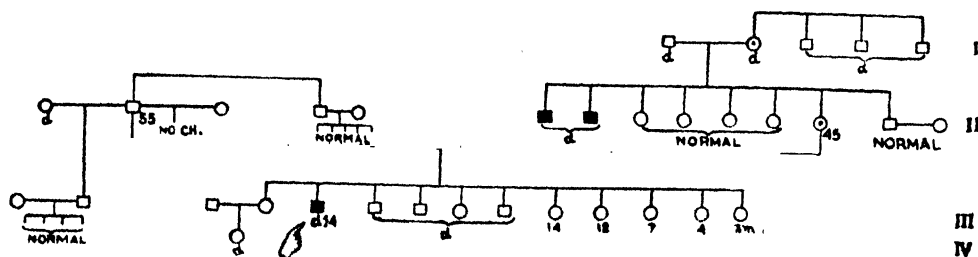
Case No. 15. K. D., male, aged 8 years, was treated privately at Ahmedabad. The patient came for medical treatment in June 1946 owing to an injury on the forehead caused by accidental fall. Bleeding continued for 5-6 days till the boy succumbed. His early history revealed ecchymosis on all parts of the body as a result of slight injury or pressure. Epistaxis was frequent at the early ages. No clinical examination of the blood was done.

The two elder brothers of the patient also died of haemophilia. The eldest had severe bleeding tendency from his very childhood. He, however, died from internal haemorrhage as a result of a fall from a wooden staircase. One of the younger brothers also died on the 6th day after his birth. In an attempt at sneezing on the 4th day of his birth he began to bleed from his nose which continued at intervals till his death 2 days afterwards.

(c) *Case from Nagpur*

Case No. 16. R. T. (Ped. 8, III 5), male, aged 14 years, was admitted on 31st March, 1952, at the Nagpur Medical College hospital with swollen knee and shoulder joints and epistaxis. He gave a history of prolonged epistaxis and severe bleeding on slight injury since 5 years of age, before which he was normal. His blood showed: bleeding time—2 min.; coagulation time—10 min. 15 sec.

PEDIGREE 8



It was found out after local enquiries by the present writer that the above boy was rushed into the above hospital on 31st December, 1953, where he expired the same day at 1.5 p.m.

It will be seen from the above pedigree that III 5 has also two affected maternal uncles (II 6 and 7) who also appear to have died from haemophilia. The latter were never treated medically. The patients belong to an illiterate weaver family. Both II 2 and his wife (II 12) described their diseases as similar to that of their son III 5. All the sisters of II 12 are said to have normal children; so also her only surviving brother. None of the other dead children of II 12 appears to be affected with haemophilia. III 6 died at the age of 7 years while the other three died within 3 years. It also transpired from the statement of II 12 that three of her maternal uncles also died at an early age. Her parental home was in Ramtek, Nagpur.

The above 16 cases were investigated by the writer as a result of a tour in Bombay, Nagpur and Ahmedabad. Their case histories were studied from the hospital records and wherever possible personal enquiries were made to complete the genealogical data.

Case No. 17. This case is shown in Ped. 9, which is from Bengal. The family has scattered about in various parts of the state and some of its members are settled in the city of Calcutta. The family was partly described by Ghosh (1942), who treated V 4 and thereby gathered some genealogical data from the father of the child. Ghosh was good enough to get the present writer introduced to the above family just before his premature death.

The child (V 4) was admitted to the Carmichael Medical College (now R. G. Kar Medical College) on 17th March, 1939, with bleeding gums and a small wound on the left margin of the tongue due to accidental biting. Echymoses was seen in the different parts of the body. His blood on 17th March showed: bleeding time—2½ min.; coagulation time—11½ min.; blood group—AB. In September 1940 the boy was again brought to Dr. Ghosh with swollen left knee joint which appeared after a fall. Then again in November 1940 he bit his tongue and came under Dr. Ghosh's treatment. At this time his blood was again examined and the following results obtained:

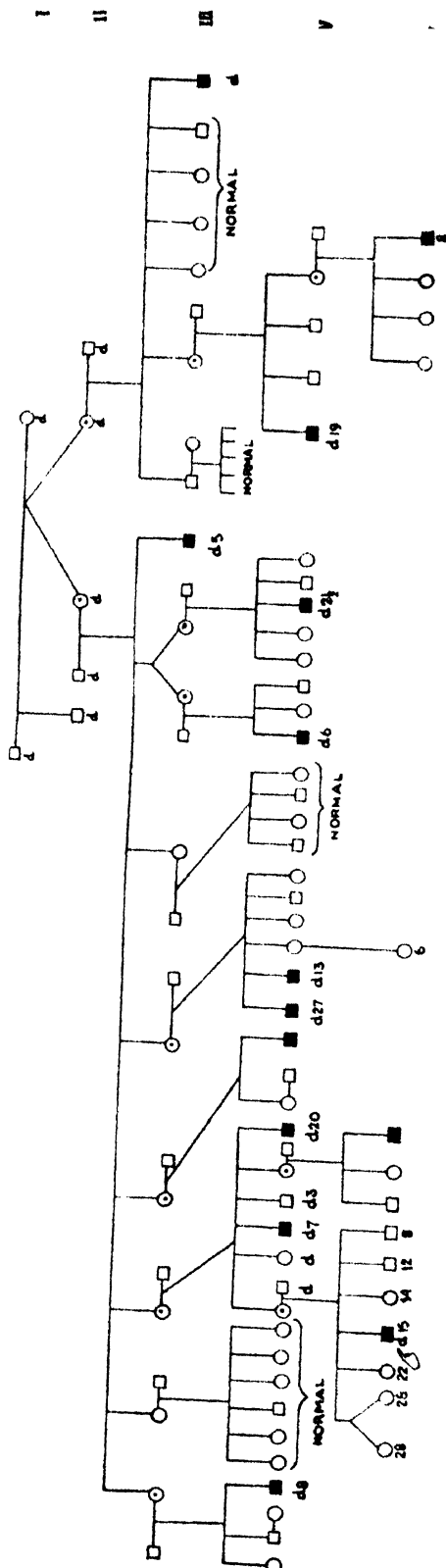
Date	Bleeding time	Coagulation time
26. 11. 40	4 min.	9 min.
29. 11. 40	4 „	9 „
18. 3. 42	3 „	15 „

Dr. Ghosh last examined the patient on 18th March, 1952, when the boy was about 6 years of age. He died in 1952.

It will be seen from the pedigree that the whole sib originated from a pair of twin sisters (II 3 and 4) one of whom has given birth to a pair of twins herself. There is also another pair of twins in the V generation who are diagnosed as monozygotic by the present writer. They are not yet married. It was difficult to trace the ancestry of the family but III 10, an old man of about 60, informed the writer that he remembers to have seen a brother of II 3 and 4, who was perfectly normal and had normal children. He lived up to an age of about 70 years. This point however could not be verified from other sources though there is no reason to doubt the statement of III 10.

It will be seen from the pedigree also that the severity of the disease is not similar in the family of the two twin sisters (II 3 and 4). II 3 shows only two normal daughters as against 6 carriers while II 4 shows only one carrier daughter against 3 normals. Haemophilia in the only child (III 17) of II 3 appears to have been correctly diagnosed. The father (II 2) was a medical man and left practically no stone unturned to save the life of his only son. While running, he accidentally hit a fish-knife and sustained a deep wound on the toes from which he bled to death.

PEDIGREE 9



The details of the children of II 4 were extremely difficult to obtain due to the reluctance on the part of the family members. At present there is only one child (V 14) who is still suffering from haemophilia. A few months ago (June 1954) he was seriously laid up with echymosis all over the body, specially the knee joints were affected and there was profuse bleeding from the gums. His tongue was paralysed, thereby preventing any intake of food with ease. His clinical details could not be secured and the case is being treated privately. The boy is now about 8 years old.

Haemophilia is almost certain in the cases of IV 22 and IV 23. Both of them used to bleed from the gums and to have echymosis and bleeding from slight injuries. The knee and elbow joints of IV 22 were severely affected and although treated privately he received the best possible medical attention available in the city of Calcutta. IV 23 died as a result of an accident. Chased by a dog he rushed into the room and while doing so he struck his head on the door lintel and died from the same injury.

It will be seen from the pedigree that in III generation there were 4 male issues of whom 2 were haemophiliacs and died of it. In the IV generation from the available information there were 19 male children of whom 9 were haemophiliacs and 10 normals. Of the 9 haemophiliacs only IV 21 is living and he is reported to have married.

Similarly there are 19 female issues of whom 7 are normals and 3 have proved to be carriers. In the V generation the present author has been able to know of 6 male issues of which 3 are haemophiliacs. None of the females of the V generation is yet married. Out of the total number of 14 haemophiliacs in the whole sib the ages at death of 10 individuals are known which gives an average longevity of 12.5 years (range: 2½ years–27 years).

The above 17 cases have been treated separately because almost all of them were initially reported from hospitals and attempts were made to gather further information on them through personal contacts. The rest of the cases will be described according to the hospitals and in the majority of cases preliminary contacts could not be established by correspondence.

(a) The V. J. hospital, Amritsar, reported to us of 5 haemophilia cases, of which two cases appeared to belong to the same individual though nothing definite can be said without a field study of the particular family. The details of these two cases are as follows:—

(i) J. (surname not recorded), male, aged 9 years, was admitted on 28th April, 1952, with epistaxis for 6 days, history of excessive bleeding from cuts and high effusion in both the knees. Clotting time—8 min. Father's name not recorded. Family history showed one brother having died of the same disease while two other brothers bleed often from minor injuries.

(ii) J. T., aged 8 years, male, was admitted on 27th April, 1953, with epistaxis for one week; haematemesis and haemorrhage into the left knee. Bleeding time—2½ min.; clotting time—8 min. 30 sec. Father's name not recorded. Family history shows one brother having died of the same disease and another suffering from it.

The similarity in the name and address of the above two patients raises firstly the doubt of their being two separate individuals. Other details are also similar to some extent. It has already occurred in the experience of the present writer that the same patient came for treatment more than once in the same hospital and has been recorded as separate cases. In such cases only field investigations can determine the true state of affairs.

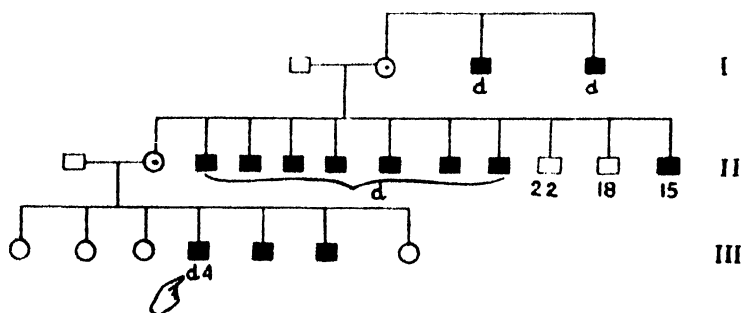
(iii) P. S., male, aged 6 years, was admitted on 6th August, 1954, with bleeding from the upper lip. He gave a history of excessive bleeding from minor injuries. The family history revealed a history of one brother also suffering from the same complaint. Two maternal-uncles were also suffering from the disease.

None of the above two cases replied to our queries. No letter was sent to (i) due to the incomplete nature of the names of the patient and his father.

(iv) I. S., male, aged 12 years, was admitted on 24th October, 1952, with bleeding gums. He showed this bleeding tendency from the age of 6 months at the slightest injury. Echymosis was very frequent all over the body and the ankles appear to have been very much affected. Whenever the milk teeth were shed he used to bleed heavily. His early history was sent to us by his father. The younger brother of the child succumbed to this disease at the age of 6 years. It was revealed from the father's letter that the boy was given blood transfusion at the hospital which did not yield any satisfactory improvement. He is now being treated privately and vitamins C and K with coagulin has been effective in his case. The youngest brother, now about 4 years old, appears to be normal. No details of the family history was sent by the father.

(v) R. C. L. (Ped. 10, III 4), male, aged 3 years, was admitted on the 31st August, 1951, with bleeding gums. The hospital records show that he was suffering from petechial haemorrhages since the age of 6 months. The father of the boy was good enough to send us some details of his family and those of his father-in-law. As regards III 4, the father informed us that in October or November 1951, while playing, the boy injured his head and began to bleed. He was rushed into the hospital where he expired the following morning. The next brother also showed symptoms of the disease from the age of 5 months. Echymosis was frequent all over the body and this symptom also appeared on the testis. The third son is also suffering from the same disease.

PEDIGREE 10



It will be seen from the above pedigree that 7 maternal uncles of the boy (III 4) have also died of this disease and the youngest (II 2) is still suffering from it. The 7 brothers died within 10 years and all were treated in a hospital, which has not replied to our queries. II 1 informs us that in all of them death followed very quickly. He also informed us of the death of two maternal uncles (I 3 and 4) of his wife from the same disease. I 1 was also contacted at the instance of II 1 but no replies were received.

(b) The Christian Medical College, Vellore, sent us records of two haemophiliacs but unfortunately none of them could be traced and our letters were returned by the Dead Letter Office.

(i) K. C., male, aged 50 years. The patient was admitted with a sudden attack of inability to talk and haemophilia into the vocal cords was diagnosed. His blood showed: clotting time—6 min. 11½ sec.; bleeding time—2 min. 1 sec.; platelets—88,800/ccm. The patient had frequent epistaxis and haematoma used to be formed at the injection spots. No family history was available.

(ii) M., male, aged 2 years. The child had a fall and injured the scalp wherefrom bleeding could not be controlled. The child was anaemic and had injured

both the liver and the spleen. His blood showed: bleeding time—2 min.; clotting time—32 min.; platelets—495,000/cmm. No family history was available.

(c) The Medical College hospital, Madras, reported of three cases of haemophilia, the details of which are as follows:

(i) M., male, aged 5 years, was admitted on April 27, 1954, with a tooth lost as a result of a fall from which he was bleeding for the last one month. Previous to it he bit his tongue and bled for 10 days. He also once bit his lip and bled from it for one month. The family history, as given by the hospital, showed an uncle had suffered from the same disease. The patient had an elder sister, who was about 7 years of age. No reply was received to our letter.

(ii) K., male, aged 30 years, was admitted on May 20, 1954, with bleeding gums. He was the only son of his parents and no such complaint is known in the family.

(iii) T. D., aged 1 year, was admitted on November 15, 1953. The other details of the patient are not available. The last two patients could not be contacted due to the absence of any address.

(d) The Irwin hospital, New Delhi, reported of only one case, the details of which are as follows:

(i) A. K. D., male, aged 14 years, was admitted on January 7, 1954, with affected joints. The coagulation time of the patient's blood has been noted as 'delayed'. No other details are known, neither was our letter replied to.

(e) The Assam Medical College hospital, Dibrugarh, reported of one case of haemophilia in an Indian Christian.

(i) P. J., male, aged 19 years, was admitted on January 18, 1953, with haematemesis. His maternal uncle was a chronic bleeder. No other details could be gathered from correspondence.

DISCUSSION

It will be seen from Table I that out of the 21 hospitals participating in our enquiry the majority, *i.e.* 13 hospitals, have not treated any case of haemophilia. The longest period involved is that of 20 years for the Mysore University Medical School followed by 10 years for the Andhra Medical College, Vishakapatnam. The G. R. Medical College, Gwalior, had no cases during the past 5 years while the neighbouring town of Indore had similarly no haemophilia cases during the past 4 years. The other 9 hospitals showing no haemophilia cases have reported only for periods varying between one to two years.

The incidence of haemophilia, however, appears to show a higher concentration along the western part of the country than that of the other regions. Starting from Amritsar on the north, we come to New Delhi to Sind (Ped. 2), to Gujarat (case No. 1, Ahmedabad cases) and then to Bombay. Out of the total number of 28 cases, excluding Bengal, 21 cases fall on the western region of the country. It is too premature to say anything definitely on this point from the present small data but a trend towards the higher incidence of haemophilia along the western region of the country is indicated.

The diagnosis of haemophilia may not be correct in all the cases. The laboratory tests on the blood are incomplete in the majority of the cases. The recent discoveries of para-haemophilia and Christmas disease necessitate detailed laboratory tests of the blood and a re-examination of the existing haemophilia cases. The majority of the pedigrees show the sex-linked recessive inheritance of the disease. The inheritance of the pedigrees 1, 2 and 3 cannot be explained due to the paucity of data. There might have been some concealment of facts in pedigrees 1, 2 and 6, while pedigree 3 is an exceptional one in having a female haemophiliac. The existing cases should be re-investigated according to the modern diagnostic methods.

There have been three marriages of haemophiliacs. In pedigree 1, II 2 has three issues, two sons and a daughter. In pedigree 5, III 10 has four issues, two sons and two daughters. In pedigree 7, I 2 has two normal sons and one carrier daughter. The last pedigree thus shows what is expected of the children of a haemophiliac father. The three haemophiliacs thus have a total number of 10 children showing an average of 3.33 children per family.

The average longevity of the haemophiliacs has been found to be 14.11 years, the maximum and the minimum being 37 years and 1 year respectively. The age at death was known in the case of 14 haemophiliacs only, of which 10 are from pedigree 9 alone. The average longevity in the latter case works up to 12.25 years. The pedigree data show that 15 haemophiliacs are still living among the affected number of 42 in 8 families. Pedigrees 3 and 4 have been excluded. The affected number of individuals increases to 52 when those from other hospitals are included. One death out of these 10* affected from other hospitals is definitely known (Amritsar case (v)). Thus when the two data are combined together it is seen that there has been at least 28 deaths in a total number of 52 affected cases.

SUMMARY

1. In order to find out the incidence of haemophilia in India a questionnaire was first of all sent to several hospitals throughout the country requesting them to inform the details of the haemophilia cases treated by them during a certain period.

2. 21 hospitals participated in the enquiry of which 13 have treated no cases of haemophilia while the rest 8 have treated haemophiliacs varying between the numbers 1 and 13. The largest number was treated by the K. E. M. hospital, Bombay, while the second largest number of 5 cases was from the V. J. hospital, Amritsar. A few cases from private medical practitioners have also been included.

3. After the addresses of the haemophiliacs were obtained attempts to contact each case were made for the purpose of collecting their family histories. A tour to Bombay, showing the largest number of cases, Ahmedabad and Nagpur was undertaken for this purpose and personal contacts with the patient or his family were made, where possible. 10 pedigrees have been collected.

4. The average longevity, based on 14 haemophiliacs, has been found to be 14.11 years. Out of the above 14 cases, 10 belong to a pedigree from Bengal, in which the average longevity works up to 12.25 years.

5. 15 haemophiliacs were living at the time of enquiry among the affected number of 42 from 8 families. The affected number increases to 52 when the total hospital data are taken into account. One death out of the latter 10 is known. Thus when the family and the hospital data are combined there have been at least 28 deaths in a total number of 52 affected ones.

6. 3 haemophiliacs are married, having a total number of 10 children.

7. A female haemophiliac child has been recorded, though the diagnosis should be confirmed by latest laboratory methods.

8. The incidence of haemophilia appears to show a higher concentration along the western regions of the country.

9. Improved clinical diagnosis is required, whereby the other two variants of the disease, namely parahaemophilia and Christmas disease, which are assumed to be allelomorphous variants of haemophilia, can be differentiated.

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* Case No. 15 has been excluded from calculation. 3 cases have been counted for Amritsar.

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STUDIES ON THE POLLUTION OF THE BHADRA RIVER FISHERIES AT BHADRAVATHI (MYSORE STATE) WITH INDUSTRIAL EFFLUENTS

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CONTENTS

	<i>Page</i>
I. Introduction	133
II. Physiographical Features of the Bhadra River near the Factories	133
III. Physico-chemical Nature of the Trade Wastes and the Effluent Diluted River Water	
(a) Pulp and Paper Mills	134
(1) Process of Paper Manufacture	134
(2) Nature of Paper Effluents	134
(b) Wood Distillation Plant Effluents	136
(c) Affected River Water	136
IV. Biotal Studies	
(1) Methods	138
(2) Distribution of Biota	
(a) Zone I—Clear Water	138
(b) Paper Mill Effluent	146
(c) Wood Distillation and Iron Works Wastes	146
(d) Zones II and III—Between Effluent Outfall and the Road Bridge	147
(e) Zone IV—Pool below Road Bridge	148
(f) Zone V—Between the Pool and the Confluence	149
V. Experimental Observations on the Effects of Paper Factory and Wood Distillation Wastes upon Fish Life	
(1) Experimental Studies on Survival of Fish	150
(2) Observations	151
VI. Observations on Fish Distribution and Condition	152
VII. General Discussion	
(a) Direct Effects	155
(b) Indirect Effects	157
VIII. Suggestions	158
IX. Summary	159
X. Acknowledgements	159
XI. References	160
XII. Appendix	162

I. INTRODUCTION

The question of affording protection to fish life has not received as much attention in India as in many progressive countries where opinions of expert fishery scientists are invited and their recommendations rigidly executed whenever damage is feared to fish life by the construction of dams and discharge of industrial wastes. Beginning with an editorial article in the *Current Science* (1938), Fowler (1939), Hora (1942), Malden (1943) and Bhaskaran (1947) have stressed the need for pollutional studies in rivers to protect fish life from the injurious effects of factory wastes and sewage contamination. Some investigations thereafter were conducted on the subject by Hora and Nair (1944), Nair (1944), Ganapathi and Alikunhi (1950) and Ganapathi and Chacko (1951). Observations on utilization of domestic wastes and sewage for fish culture were described by Bose (1944) and Fowler (1944). Data from mainly a sanitary engineering and public health point of view were collected by Modak (1938) and Seth and Bhaskaran (1950, 1952 and 1952a) the latter making some observations also upon the possible conversion of such wastes into valuable by-products.

The present study was conducted during 1945 under the guidance of Dr. B. S. Bhimachar, the then Fisheries Officer, Mysore State, as a result of an enquiry by the I.C.A.R. to the State Government. A detailed report was prepared in 1946, but it was not possible to compile it in the form of a scientific paper till now, although a short account was presented at the Indian Science Congress Session (1946) by Bhimachar and David (1946).

II. PHYSIOGRAPHICAL FEATURES OF THE BHADRA RIVER NEAR THE FACTORIES

The Mysore Paper Mills and the Mysore Iron and Steel Works are situated on the left bank of the Bhadra river in the township of Bhadravathi in Shimoga district. The hilly and forest catchment of the river Bhadra, which together with the Thunga forms the Thungabhadra, is about 950 sq. miles with an annual rainfall of about 150 inches, which is variable between 46 and 202 inches. The total length of the river is only about 115 miles, covering elevations between 5,540 and 2,000 feet above m.s.l. and hence is sub-montane in character. It has a perennial flow with a maximum discharge of about 90,000 cusecs during the height of the monsoon and a minimum flow of 75 cusecs during the driest part of the year, i.e. May. The course of the river is tortuous, and its bed is carved out of hard granite rocks forming precipitous banks, ravines and rapid vortices. At Bhadravathi, the river enters comparatively the drier part of Shimoga district with an undulating countryside and is mainly rocky with occasional sandy and gravelly patches. The right bank (being lower than the left) is subjected to overflows whenever there is a moderate rise of water. Exposed sandy part of the bed is generally covered by a scrubby and bushy vegetation. There are numerous broken up channels, quiet pools and riffles fit enough to shelter a good fish population in the stretch of the river near Bhadravathi. The river is only 300 to 525 yards wide without any appreciable sized stream bringing into it a fresh supply of water below Bhadravathi.

A low masonry weir (sketch map) across the river serves to maintain sufficient depth for the intake of water by the Iron and Steel Works. For avoiding contamination by the pollutants, the outfall of the Paper Mill effluents is led below the weir. Wood distillation wastes and washings from the Steel Works are, however, drained below the railway bridge, some half mile below the first discharge head. Between the weir and the road bridge, for a distance of nearly a mile, the flow in the river is restricted to the right bank during the dry months, exposing part of the river bed on the left. Presence of a large reservoir-like pool of about half sq. mile in area below the road bridge observed at the time was of some importance

in this study as it acted as a septic-cum-recovery pool for the combined effluents. A small pool (cul-de-sac) at the mouth of a dried up minor stream (sketch map) on the right was often found to shelter affected fish.

III. PHYSICO-CHEMICAL NATURE OF THE TRADE WASTES AND THE EFFLUENT DILUTED RIVER WATER

The Mysore Iron Works commenced functioning in 1924 when smelting of iron ore from the nearby quarries with wood charcoal specially manufactured in an accessory wood distillation plant was undertaken. Later, several new factories, e.g. a paper mill, steel rolling mill, cement factory, fire bricks factory, etc., were set up close to it. During the war years the problem of water supply was considerably aggravated by the fact that the river water began to be contaminated by effluent discharges of the paper mill. Though there do not appear to have been complaints originally, only after the establishment of the paper mill (sometime during 1939) the pollution of the water was brought to the notice of the Government owing to the bad odour and discoloration. Thereafter, a few tests were carried out by the State's Chemical Examiner, but no effective remedial measures appear to have been taken. During investigations in 1945 the work was handicapped for want of a chemist and laboratory facilities for water quality tests, such as determination of B.O.D., etc. Hence the author has relied mainly upon a few chemical tests which he himself could undertake in the laboratory of the Iron and Steel Works.

(a) *Pulp and Paper Mills*

(1) *Process of Paper Manufacture*

The Mysore Paper Mills manufacture various grades of writing and printing paper from bamboo pulp. Its production during 1945 was about 12 tons per day from an average of 25 tons of dried raw bamboo. Manufacture of pulp is by the 'Sulphate' process. Well-dried bamboo chips are fed into three digesters followed by a solution of sodium salts such as caustic soda and sodium sulphate together with calcium carbonate. Under high pressure cooking with steam at 160°C., sodium-bisulphide is produced which together with caustic soda softens the fibrous tissue separating the cellulose pulp from lignins and pectins. The 'spent lye' or a mixture of waste chemicals which is a black syrupy liquid is evaporated, dried and incinerated with an addition of sodium sulphate. Most of the lignin is burnt in this process partly supplying the combustion heat. The resulting ash is dissolved in water to form the 'green liquor', which after causticizing with lime produces the 'white liquor' for re-use. Recovery processes are usually adjusted to keep pace with production of pulp, but in emergencies such spent lye may be let out into the general wastes causing the fish mortality reported often from sites of many paper mills in India. The amounts of chemicals and raw materials utilized and the products obtained per each production day (during 1945 and during 1955) are as in the following Table.

During 1945 one ton of chlorine was being produced to obtain the required quantity of sodium-hypo-chlorite for bleaching pulp and 1.5 tons of alum and 1 ton of rosin were also employed in the paper machine for sizing. There appears to be a proportionate increase of nearly twice the above figures during 1955.

(2) *Nature of Paper Effluents*

The waste liquor from the digesters contains the original cooking solution in combination with nearly all the cellulose constituents of the bamboo and a complex mixture of sulphur compounds with organic derivatives like mercaptans, ligno-sulphonates, fatty and resin acids. Some amount of formic and acetic acids are

Raw materials	1945	1955	Products	1945	1955
	Tons	Tons		Tons	Tons
Bamboo	25	50	Pulp	10	24
Rags	3	3	Paper	12	20
Waste paper	2.5	3	Cardboard	0.5	1
Caustic soda	1	3	Effluents	Mil. gals.	Mil. gals.
Sodium sulphate	3	6		2	3.5
Lime stone (calcium carbonate)	5	14			
China clay	2	4			
Sodium chloride	2	2			
	Mil. gals.	Mil. gals.			
Water	3	4			

also present. Untreated alkali and alkali sulphides also go into solution. A high percentage of *fermentable sugars* are also found in such wastes, but in bamboo pulp wastes, this can be considerably higher. The relief gases which condense contain also the bad smelling mercaptans, sulphur-di-oxide, di-methyl-sulphide, methyl alcohol, ammonia, etc., with traces of turpentine and similar aromatic oils. Chlorine utilized for bleaching pulp is also washed off mostly as chlorides. As chlorine readily forms compounds with organic substances, a number of such compounds may be present in the effluents. The total rate of discharge of effluents into the river works out at 1,388 and 2,430 gallons per minute respectively during 1945 and 1955. Considering that the flow of water in the river at a modest estimate is 72-80 cusecs (472-505 gallons) per second in April and May, the completed dilution of the paper effluents in the river works out at between 185 and 208 times in 1945 and 106 and 120 times in 1955. These dilutions are for the driest months of the year in April-May after stabilized conditions prevail (following the previous monsoon floods) for about seven months.

The mixed effluent originating from the various plants is usually brownish, but is variable in colour, smell and other properties, becoming light brown, green or yellow, but is always ill-smelling, pungent or irritating, depending upon the nature of washings. The Chemical Examiner's reports show the following fluctuating values :—

	Minimum	Maximum
	p.p.m.	p.p.m.
Total solids	770	1,460
Suspended solids	180	580
Dissolved solids	590	880
Oxygen absorption in 24 hrs.	32	83
Chlorine	80	200
Sulphide as H ₂ S	40	47

In samples of undiluted effluents, suspended particles settle to about $\frac{1}{4}$ of the graduated cylinders. Allowing to settle for 24 hours or boiling did not destroy the smell nor colour of the liquor materially. Though all fibres were removed by filtration, filtrate did not lose its colloidal nature due to suspension of sulphur freed from sulphides by oxidation. It is obvious from the table that the values of dissolved solids are much higher than suspended solids.

(b) *Wood Distillation Plant Effluents*

Smelting iron ore with *wood-charcoal*, a rare process, is profitable in Mysore on account of the abundance of forest wood. Charcoal is manufactured in the ancillary wood distillation plant of the Mysore Iron and Steel Works in airtight iron retorts where uniformly cut raw wood is subjected to slow destructive distillation. Vapours are condensed into the valuable pyroligneous liquor which is subjected to solvent extraction and fractional distillation to isolate acetic acid, wood naphtha, wood tar, methyl alcohol, methyl acetone and formaldehyde. The average quantities of the above products are as follows :—

Amount of raw wood used	..	100	tons	(per day)
Charcoal produced	..	25	"	..
Pyroligneous liquor	..	10,000	gallons	..
Acetic acid	..	1.5	tons	..
Wood naphtha, crude	..	250	gallons	..
Wood tar	..	2	tons	..
Methyl alcohol	..	100	gallons	..

Amount of effluents generated—10,000 gallons per day.

In addition to the above components, unrecoverable pyroligneous liquor is let out frequently when acidity falls below 2 per cent.

The effluent which is discharged into the river is a brown liquid with a pyrogenous smell holding minute quantities of phenolic bodies and tar particles in suspension and contains about 0.3 per cent acetic acid. Its estimated flow into the river was about 7 gallons per minute. The flow being slow in the open meandering drain, much of the suspended particles tend to settle to the bottom, forming an impervious sticky bottom of hard tar.

(c) *Affected River Water*

The effluents from the pulp and paper mills are led into a small open settling tank which, having silted up, served no useful purpose at the time of these studies. Overflow from this swampy tank was then led into a 2 ft. diameter pipe which carried it by gravity to the river's bank for a distance of nearly a mile, to discharge with considerable force into the river below the weir (sketch map). By an eight feet fall and the presence of caustic soda and other alkaline mixtures, a huge amount of soapy froth was being continually generated at this spot. The characteristic sulphate mill smell of mercaptans was very strong in the vicinity. A dirty greenish black sub-soil of 6-8 ft. depth covered the river's bed at the outfall where foul-smelling gases could be observed bubbling up. Pulp with precipitated gritty chemical substances formed cardboard-like layers, which constantly peeled off from the dry river margin. The effluents imparted a dark brown colour to the water close to the left margin, clearly demarcating the polluted waters on the left bank from unpolluted water until admixture took place almost completely close below the road bridge. The discoloration persisted in the Bhadra till its confluence with the Thunga.

As dilution of the paper effluents increased in the water, a dark soft ooze became perceptible at the bottom, blanketed by colonies of whitish fluffy fungus-like higher bacteria and *Tubifer* worms. A good amount of pulp fibres and silt were also caught in these colonies which gradually built up a loose anaerobic substratum. Roughly, this part of the river may be designated as the paper wastes' pollutional area, though somewhat complicated by the addition of wood distillation wastes part of the way below the railway bridge.

In the following table, results of several chemical tests that were carried out by the author during summer months of April and May are given. Three series of tests were conducted on samples collected at stations 2 and 3 and one series at 1, 4

and 5 on different days spread over a month. Where fluctuations were high, both the maximum and the minimum values noticed are given (on different days), but when low, only averages of the readings are noted. The Chemical Examiner's reports indicate that the river water has a B.O.D. of 0.5 p.p.m. in 12 hours and 17.5 p.p.m. in 24 hours at 65°F. which is quite high. According to Gehm (1948), 550-750 lbs. of B.O.D. are discharged per ton of pulp produced in America, and this is mainly due to the sugars present in the wood used. These values are likely to be much higher in bamboo than in American forest woods. Repeated tests did not reveal even traces of ammonia in samples of water collected for a mile below the paper effluent outfall, showing the low nitrogen content.

TABLE I

(All values given are parts per million--p.p.m.)

Station	Dissolved oxygen	Dissolved carbon-dioxide	Dissolved chlorine	Dissolved hydrogen sulphide
1. Unpolluted waters (above weir)	11.2	4.84
2. Above railway bridge. Polluted with paper mill effluent	8.65 to 10.00	5.48	10.64 to 19.00	8.00 to 15.2
3. Between bridges. Polluted with both paper mill and wood distillation plant effluents ..	8.4 to 9.36	4.7	7.55	2.58
4. Below road bridge	8.08	3.06	7.80	2.04
5. Close to confluence of the Bhadra with the Thunga	10.24	4.2	9.36	0.82

From an inspection of the foregoing values it is observed that the river being a cascading type, slightly higher D.O. in surface waters is not unusual. It is only slightly reduced when the paper effluents mix with the river water. It is also not too low in observation points downstream, indicating thereby that known lethal limits of oxygen depletion are perhaps not generally reached in the waste-diluted portion of the river. Amount of CO₂ in river water does not seem to be greatly affected, but an intense photo-synthetic activity by chlorophyll bearing phytoplankton and higher flora cannot be entirely ignored in station 4, where slightly lower values were observed. Lethal limits of CO₂ saturation are not often reached but fish are known to be highly resistant to free CO₂, and only prolonged exposures to 50-100 p.p.m. of free CO₂ dissolved in water prove lethal to most fish (Doudoroff and Katz, 1950).

It is also seen that free Cl₂ in undiluted paper wastes varies between 80 and 200 p.p.m. (Chemical Examiner's report). A value of as much as 9.36 p.p.m. in river water 11 miles below may be an interesting exception due to excessive hypochlorite washings. It may be mentioned here that these values are many times higher than the known lethal doses of 1.00 p.p.m. Since several sulphur compounds are utilized in the paper mills producing complicated mixtures, only the intermediary total sulphide values were determined. A total sulphide value between 40 and 47 p.p.m. in the raw paper effluent is variable on dilution only between

8:00 and 15:20 p.p.m. in the river water and indicates that accumulation of sulphide occurs in the stream. Thereafter it gradually diminishes to 0.82 p.p.m. lower down. Precipitation of sulphur compounds invariably takes place and production of H_2S from the soft sub-stratum also occurs in appreciable amounts as evidenced by the numerous bubbles released.

IV. BIOTAL STUDIES

(1) *Methods*

For purposes of biotal study, the several areas were designated roughly as follows :—

- (1) Zone I—*Clear Water Area*—Comprising the uncontaminated river stretch above the weir.
- (2) Zone II—*Paper Mill Polluted Area*—The river stretch between the paper wastes outfall and the railway bridge.
- (3) Zone III—*Pollutional Area* of both the paper mill and wood distillation plant wastes—Between the railway bridge and the road bridge.
- (4) Zone IV—*Septic-cum-Recovery Area*—Large pool some distance below road bridge and connected channels.
- (5) Zone V—*Recovery Area*—Nine miles stretch below the large pool to the confluence of the Bhadra with the Thunga.

Besides the above, the undiluted paper mill and wood distillation waste channels have been considered separately for biotal studies together with their connected stagnant pools.

For a better understanding of the nature of biota (excluding fishes which are dealt with in a separate chapter) and their distribution, comprehensive collections of flora and fauna were made both qualitatively and to a lesser degree quantitatively. Floating and swimming organisms were collected by a circular close-meshed hand-net with a bucket provided at the apex. For deeper areas, an ordinary ring plankton net was used with a long hauling-in cord. Ooze along the margin up to a water depth of 2 ft. was collected by pushing a semi-circular-shaped hand-net provided with teeth along its diameter, and thus well adapted for scraping the surface. Careful attention was paid for collecting 'epiphyton' (organisms coating submerged objects) where ordinary scalpels and watch glasses were used. From inaccessible pools of over 4 ft. depths, sub-surface bottom tows were made by nets mounted on runners and the deep bottom sludge by a toothed triangular dredge similarly mounted, both provided with long cords for hauling. The collection equipment was made locally according to Reighard's designs (*vide* Ward and Whipple, 1945).

As far as possible, uniformly identical hydrological conditions, such as currents, depths, sub-strata, etc., were chosen for comparison from one part of the river to the other while collecting organisms. Bottom sludge was washed in glass jars and macro-fauna hand-picked and counted. In case of specific indicator organisms, their counts were made to compare their density in each square foot of water not exceeding 5' in depth at suitable intervals. As these studies at best could only be relative, the intensity of distribution of the various groups are expressed on lines usually indicated in such studies and denoted as abundant, very common, common, fairly common, infrequent, rare and absent.

In Table II, the list of organisms provisionally identified up to the genera or family is given for easy reference.

(2) *Distribution of Biota*

(a) *Zone I—Clear Water.*—A reference can be made to Table II where the several organisms found in the unpolluted natural water are listed. Peculiarities

TABLE II

Distribution of biota in the stretch of the Bhadra investigated

*****—Abundant
 *****—Very common
 *****—Common
 ***—Fairly common
 **—Infrequent
 *—Rare
 ab —Absent
 .. —Not observed. (Probably absent)

Genera or Family	Zone I— Clear water	Paper effluent overflow	Wood distillation waste	Zones II and III— Paper effluent in river	Zone IV— Septic-cum- recovery area	Zone V— Recovery area	Remarks
1	2	3	4	5	6	7	8
FAUNA							
Protozoa	****
Actinopoda
Amoeba
Arcella
Diffugia
Centropyxis
Euglypha
Nebela
Hydrospira
Phryginella
Chaetia
Didymium
Tracheilus
Colpidium
Paramacium
Sarcocystis
Vorticella
Mastigophora (5 species)

TABLE II—(Contd.)

Genera or Family	Zone I— Clear water	Paper effluent overflow	Wood distillation waste	Zones II and III— Paper effluent in river	Zone IV— Septic-cum- recovery area	Zone V— Recovery area	Remarks
1	2	3	4	5	6	7	8
<i>Porifera</i>							
<i>Trichostrongyloidea</i> ?	**	ab	ab	ab	***	**	
<i>Ephydra</i> ?	**	ab	ab	ab	**	*	
<i>Ooenterata</i>							
<i>Hydra</i>	**	ab	ab	**	*****	*	
<i>Platyhelminthes</i>							
<i>Turbellaria</i>							
<i>Catenula</i> ?	***	ab	ab	*****	*****	**	
Unidentified	*****	*	
<i>Nemathelminthes</i>							
<i>Nematoda</i>							
Unidentified (a)	**	**	*	
Unidentified (b)	ab	***	***	*	
<i>Rotatoria</i>							
<i>Notommatia</i>	****	ab	ab	*	*****	**	
<i>Dinocystidae</i> (2 sp.)	****	ab	ab	ab	*****	**	
<i>Conochilus</i>	*****	ab	ab	ab	*****	**	
<i>Monostyla</i>	*****	**	*****	*	
<i>Diplois</i>	*****	**	*****	*	
<i>Notus</i>	*****	..	*****	**	
<i>Meliceria</i>	*****	..	*****	**	
Unidentified (a)	*****	*	
Unidentified (b)	ab	**	*****	*	

TABLE II—(Contd.)

Genera or Family	Zone I— Clear water	Paper effluent overflow	Wood distillation waste	Zones II and III— Paper effluent in river	Zone IV— Septic-cum- recovery area	Zone V— Recovery area	Remarks
1	2	3	4	5	6	7	8
<i>Chaetopoda</i>							
<i>Dero</i> ..	ab	..	*	***	***	*	
<i>Tubifer</i> ..	*	..	*	*****	****	*	
<i>Stylaria</i> ..	ab	..	**	*****	****	**	
Unidentified spp. (terrestrial)	***	ab	ab	ab	*	**	
<i>Mollusca</i>							
<i>Lymnaea</i> ..	*	ab	ab	*	****	**	
<i>Vivipara</i> ..	**	ab	ab	*	*****	**	
<i>Melania</i> ..	**	ab	ab	*	*****	*	
<i>Indoplanorbis</i>	**	ab	ab	ab	*****	*	
<i>Pleurocera</i> ?	***	ab	ab	ab	***	*	
<i>Unio</i> ..	**	ab	ab	*	****	*	
<i>Corbicula</i> ..	*	ab	ab	ab	**	*	
<i>Hirudinea</i>							
Unidentified	**	..	Parasitic on gastro- pod.
<i>Copepoda</i>							
<i>Diaptomidae</i> (several spp.)	**	ab	ab	ab	*****	**	
<i>Cyclopidae</i> (several spp.)	***	ab	*	**	*****	**	
<i>Cladocera</i>							
<i>Sididae</i> (several)	***	ab	ab	ab	*****	***	
<i>Daphnidae</i> (several)	***	ab	ab	***	*****	**	
<i>Bosminidae</i> (several)	***	ab	*	***	*****	**	
<i>Macrothricidae</i> (several)	**	ab	*	ab	*****	*	
Unidentified spp.	**	ab	*	ab	*****	*	

TABLE II—(Contd.)

Genera or Family	Zone I— Clear water	Paper effluent overflow	Wood distillation waste	Zones II and III— Paper effluent in river	Zone IV— Septic-cum- recovery area	Zone V— Recovery area	Remarks
1	2	3	4	5	6	7	8
<i>Ostracoda</i>							
Unidentified spp. (<i>Cypris</i> ?)	*	ab	ab	ab	***	*	
Burrowing form	..	ab	ab	ab	***	..	
<i>Decapoda</i>							
Prawns	***	ab	ab	ab	*	*	
Crabs	**	ab	ab	ab	ab	ab	
<i>Hydracarina</i>	*	ab	ab	ab	**	..	
<i>Insecta</i>							
<i>Orthoptera</i> (Acridiids) <i>Scelermina</i>	ab	ab	ab	****	****	**	On damp margins.
<i>Dermaptera</i> (Forficulids)	*	**	**	****	*****	*	
<i>Plecoptera</i> — <i>Perlaria</i> spp.	**	ab	ab	ab	ab	*	
<i>Plecoptera</i> (Ephemerids) <i>Ephemeru</i>	**	ab	ab	ab	ab	*	
<i>Odonata</i> — <i>Anisoptera</i> (Dragon fly nymphs)	**	ab	**	****	*****	***	
<i>Odonata</i> — <i>Zygoptera</i> (Damselfly nymphs)	**	ab	ab	ab	*****	**	
<i>Hemiptera</i> — <i>Hydrometridae</i> (Skaters)	*	ab	ab	ab	*****	*	
<i>Hydrobatidae</i>	**	ab	ab	ab	*	..	
<i>Nauoridae</i>	*****	..	
<i>Belontioidae</i>	*	ab	*	*	*****	*	
<i>Nepidae</i> — <i>Laccotrepes</i>	**	ab	**	*	*****	**	
<i>Nepidae</i> — <i>Ranatra</i>	*	ab	ab	ab	***	*	
<i>Notonectidae</i> — <i>Anisops</i>	*	ab	**	*	****	**	At least 2 spp. one <i>Micronecks</i> ob- served.

TABLE II—(Contd.)

Genera or Family	Zone I— Clear water	Paper effluent overflow	Wood distillation waste	Zones II and III— Paper effluent in river	Zone IV— Septic-cum- recovery area	Zone V— Recovery area	Remarks
1	2	3	4	5	6	7	8
<i>Corixidae</i>	*	ab	*	*	****	**	
<i>Trichoptera</i> (several genera)	**	ab	ab	ab	****	**	
<i>Lepidoptera</i> — <i>Pyraustidae</i> ?	**	..	
<i>Coleoptera</i> — <i>Haliptidae</i> ..	**	*	**	**	****	..	
<i>Dytiscidae</i> (several) ..	***	***	**	***	****	***	
<i>Gyrinidae</i>	***	..	**	*	****	*	
<i>Hydrophilidae</i>	**	ab	**	*	****	**	
<i>Hymenoptera</i> (several spp.)	**	**	****	****	****	**	
<i>Diptera</i> — <i>Psychodidae</i> (Sand flies)	**	ab	****	****	****	..	
<i>Dixidae</i>	***	ab	**	****	****	*	
<i>Culicidae</i> (several spp.)	*	****	****	****	****	**	
<i>Chironomidae</i>	ab	****	****	****	****	****	
<i>Simuliidae</i>	ab	ab	**	****	**	*	
<i>Leptidae</i>	ab	ab	****	**	****	**	
<i>Syrphidae</i> (<i>Eristalis</i> and several others)	ab	ab	****	***	****	*	
FLORA							
<i>Eubacteriales</i> -Bacteria (Unicellular) ..	ab	ab	**	***	***	***	
<i>Chlamydoobacteriales</i>							
<i>Crenothrix</i>	ab	ab	ab	*****	***	**	
<i>Beggiola</i>	ab	ab	ab	*****	***	**	
<i>Thiobacteria</i> (several types) ..	ab	ab	ab	*****	*****	*****	
<i>Fungi</i> (<i>Leptothrix</i> ?) and several varieties	ab	ab	***	***	***	*	

On damp margins.

TABLE II—(Contd.)

Genera or Family	Zone I— Clear water	Paper effluent overflow	Wood distillation waste	Zones II and III— Paper effluent in river	Zone IV— Septic-cum- recovery area	Zone V— Recovery area	Remarks
1	2	3	4	5	6	7	8
Mycophyceae							
<i>Spirulina</i>	***	***	****	*****	..	
<i>Clathrocystis</i>	ab	**	ab	*****	..	
<i>Oscillatoria</i>	*****	*****	*****	***	..	
<i>Nostoc</i> ..	*	***	*****	*****	**	..	
<i>Anabaena</i> ..	*	**	..	
<i>Cylindrocapsa</i>	***	..	*****	..	
<i>Batrachospermum</i>	***	**	..	**	..	
Bacillariophyceae							
<i>Cyclotella</i>	***	****	***	..	
<i>Pleurosigma</i>	ab	***	***	*****	..	
<i>Diploneis</i>	ab	***	***	*****	..	
<i>Stauroneis</i>	ab	ab	ab	***	..	
<i>Navicula</i>	*****	***	*****	*****	..	
<i>Pinnularia</i>	*****	*****	*****	*****	..	
<i>Gomphonema</i>	*****	*****	*****	*****	..	
<i>Nitzschia</i>	ab	ab	**	***	..	
<i>Asterionella</i>	ab	ab	ab	*****	..	
<i>Tabellaria</i>	ab	***	***	*****	..	
<i>Synedra</i>	*****	..	
<i>Fragilaria</i>	***	*****	..	
<i>Cymbella</i>	ab	ab	ab	*****	..	
<i>Mastogloia</i>	***	..	*****	..	
<i>Achnanthes</i>	ab	ab	ab	*****	..	
<i>Coconeis</i>	ab	ab	ab	*****	..	
<i>Rhopalodia</i>	***	..	**	..	
<i>Eunotia</i>	***	

TABLE II—(Concluded.)

Genera or Family	Zone I— Clear water	Paper effluent overflow	Wood distillation waste	Zones II and III— Paper effluent in river	Zone IV— Septic-cum- recovery area	Zone V— Recovery area	Remarks
1	2	3	4	5	6	7	8
<i>Chlorophyceae</i>							
<i>Pandorina</i> ..	***	ab	ab	**	****	*	
<i>Eudorina</i> ..	***	ab	*	..	****	*	
<i>Volvox</i> ..	***	ab	ab	ab	****	*	
<i>Tetraspora</i> ..	***	**	***	..	
<i>Gosmarium</i> ..	***	ab	****	ab	**	*	
<i>Zygnema</i> ..	***	ab	..	ab	****	..	
<i>Matigeotia</i> ..	***	ab	ab	ab	****	..	
<i>Staurastrum</i> ..	**	ab	ab	ab	****	..	
<i>Cylindrocapsa</i>	****	..	
<i>Closterium</i> ..	***	ab	**	ab	***	..	
<i>Ankistrodesmus</i>	..	ab	ab	*	ab	**	
<i>Denticium</i>	ab	ab	ab	****	*	
<i>Spirogyra</i> ..	****	ab	ab	ab	****	***	
<i>Eustrum</i> ..	***	ab	ab	ab	****	..	
<i>Ulothrix</i> ..	****	ab	ab	ab	****	*	
<i>Cladophora</i>	****	ab	ab	ab	****	..	
<i>Chaetophora</i>	****	ab	ab	ab	****	****	
<i>Chara</i> ..	****	ab	ab	ab	****	****	
<i>Euglena</i> (several)	****	ab	****	ab	****	****	
<i>Chlamydomonas</i>	****	ab	****	***	****	*	
<i>Hydrodictyon</i>	****	ab	***	**	****	*	
<i>Chaetophaeidium</i>	**	..	*	
<i>Trichonema</i>	*	
<i>Microsterias</i>	*	
<i>Macrophytes</i>							
<i>Lemna</i> ..	*	ab	ab	ab	***	..	
<i>Marattia</i> ..	*	ab	ab	ab	****	*	
<i>Hydrilla</i>	ab	ab	ab	****	*	
<i>Vallisneria</i>	ab	ab	ab	****	..	
<i>Apogoneton</i>	..	ab	ab	ab	****	..	
<i>Potamogeton</i>	..	ab	ab	ab	****	..	
<i>Eichhornia</i>	..	ab	ab	ab	****	*	
<i>Polygonum</i>	..	ab	*	**	****	*	
<i>Jussaea</i> ..	*	***	*	*	****	***	
<i>Cyperus</i> ..	**	****	*	***	****	***	
<i>Typha</i> ..	**	****	*	***	****	***	
<i>Ipomea</i> ..	*	*	*	*	****	*	
<i>Ottelia</i> ..	ab	ab	ab	ab	***	ab	

observed in their distribution are discussed with reference to the polluted areas in the ensuing paragraph.

(b) *Paper Mill Effluent*.—The raw effluent is not entirely devoid of organic life (Table II), though there appears to be an absence of bacterial life due to the bactericidal action of excessive alkalies, chlorine, hypochlorite and sulphurous acid. A few colourless Ciliata such as *Colpidium*, *Chaenia*, *Paramaecium*, insect larvae such as those of *Culicids*, *Chironomids* and *Haliplus* and two small adult *Dytiscid* beetles constituted the animal life of this toxic water in the aged stagnant pools where no fresh effluent admixture was taking place. A few Diatoms, namely *Navicula*, *Pinnularia*, *Gomphonema* and *Fragillaria*, and some Euglininae and Cyanophyceae, like *Euglena*, *Oscillatoria*, *Nostoc*, *Spirulina* and *Batrachospermum*, represented the aquatic plant life. Presence of *Typha* and other grasses on the margins of the overflow tank, together with a thick growth of some other weeds, showed that the rooted plants probably do not die out in the effluent fed water.* A large number of frogs inhabited the edges of the pool indicating thereby that the effluent is not toxic to them. No tadpoles were, however, found in the area.

(c) *Wood Distillation and Iron Works Wastes*.—Absence of any animal or plant life in the flowing wood distillation waste liquor, mixed with sulphuric acid from the steel plant, is not surprising as it is highly acidic. But the fluffy, brownish floating scum formed on the surface of the stagnant sandy pools in the river bed consisted of a dense growth of Fungi conglomerated with various kinds of Bacteria, Diatoms, etc. Of the latter, *Rhopalodia*, *Mastogloia*, *Eunotia* and of the Desmids, *Cosmarium*, *Merismopaedia*, etc., may be mentioned. Some Protozoans, *Tracheilus*, *Sarcocystis* and *Paramaecium*, were found attached to the wet undersurface of the flocculent substance together with Rotifers, *Monostyla*, *Diplois*, *Noteus* and *Meliceru*. *Oscillatoria* and *Nostoc* were also found in this surface scum. Though the raw liquor is highly toxic, due perhaps to the formation of complicated sugars by polymerization of numerous aldehydes present (on prolonged exposure to atmosphere), favourable conditions for their growth are created which support an aerobic life. A dark ooze at the bottom consisting of fine tar particles supported a fairly rich Bacterial and Protozoan life of the anaerobic type. These included, among Bacterial group, unicellular Bacteria and the higher group of both septate and non-septate 'Fungi' *Leptothrix*, and amongst the Protozoans mainly the shell forming varieties *Sarcodina*, *Diffugia*, *Actinopoda*, *Arcella*, *Euglypha*, *Centropyxis*, etc. A number of colourless Infusorians, namely *Sarcocystis*, *Paramaecium* and a variety of colourless Flagellates, were also observed.† Raymond and Lindmann (1942) have stated that colourless Flagellates can thrive under highly anaerobic conditions. Clusters of 'rat-tailed maggots' of *Eristalis* fly within the sludge are further evidence of a high degree of anaerobicity of the bottom. According to Campbell (1939) these maggots thrive in the short zone of active decomposition where anaerobiosis flourishes along with anaerobic Protozoa. Such conditions are always associated with an absence of Fungi and green plants. Larvae of *Psychoda* fly and *Leptid* pupae (snipe flies) were also found in abundance in this area with the adults hovering on the damp edge. Two species of Copepoda and one of Cladocera were collected only occasionally in the aged pools. Three to four species of Diatoms were also observed in the ooze. It is obvious from the above that a certain amount of breaking up of organic components, e.g. by Bacterial activity, upon phenols and polymerization of organic acids, may occur in the wood distillation

* Author has since noticed a fairly good vegetable gardening practised by direct utilization of paper effluents at Dehri-on-Sone in Bihar, the raw effluents serving both watering and manuring purposes.

† Well corked tubes in which ooze samples were kept without fixative showed living colourless Infusorians after a lapse of three months. The original phenolic smell still persisted as obviously no chemical action had taken place. Many generations of Infusorians must have lived in the tube during this period.

effluent on long exposure to atmosphere and sunshine. In course of time this begins to support both aerobic and anaerobic life. This does not involve any dilution.

(d) *Zones II and III—Between Effluent Outfall and the Road Bridge.*—The region between the paper effluent outfall and the road bridge is treated as a separate sub-unit for purposes of discussion here (unless otherwise stated) as the effects attributable to wood distillation effluents alone are obscured owing to its broken streamlets mixing with the already paper waste-diluted water of the river at several points. This stretch is characterized by a dense growth of sheath-forming Bacteria (both septate and non-septate) of the *Sphaerolitus* (*Crenothrix*) and *Beggiota* types. At first sight their colonies may be mistaken for tufts of dirty cotton flocs or pulp and even 'flocculent deposition of certain salts'. It was further seen that several types of unicellular *Thiobacteria* and *Eubacteria* are also dominant in this stretch storing sulphur compounds in their cell structures. This fact is of importance as constituting one of the most potent hazards to fishes which feed upon the bottom muck.

Thick masses of Tubificid colonies were the next important animal group. These aquatic Chaetopodans, mostly *Dero*, *Stylaria* and *Tubifex*, were found thriving side by side with fluffy bacterial colonies. Raymond and Lindmann (*op. cit.*) have found that their presence is a sure sign of high degree of anaerobiosis and that they have a capacity to split water into Oxygen and Hydrogen for their survival. Their gills further assist the process of oxygen absorption. The extent of projection from their tubes indicates the relative oxygen depletion in a bacterially active substratum. Though well over $\frac{1}{2}$ of their bodies were found to project outside their muddy tubes in the area near the railway bridge, hardly $\frac{1}{8}$ part of their bodies were seen exposed further down 8 miles below, where a certain amount of recovery had taken place. Of these, *Stylaria* with its long proboscis was predominant in the area above the railway bridge and *Dero* more so below. Their decreased density lower down was not perhaps so much due to any improved water conditions but to the presence of numerous minnows feeding voraciously upon them (see p. 141).

Terrestrial Oligochaetes were absent in marginal areas even slightly affected by polluted portion of the river flow indicating their great sensitivity towards percolated effluent waters. These reappeared only two miles below the first outfall.

Protozoa generally increased in density but showed a decided decrease in *Paramaecium* and *Chaetia*; whereas *Arcella*, *Nebela*, *Phryginella*, *Colpidium*, etc., were recorded from paper wastes polluted region, *Actinopoda*, *Euglypha*, *Tracheilus* and *Sarcocystis* were found in the area where there was an admixture of wood distillation wastes as well. Turbellarians and free swimming Nematoda were found in the first part of the stretch above the railway bridge but were absent below it in the wood distillation waste affected part. Though a few Rotifera, i.e. *Monostyla*, *Diplois*, *Noteus* and two species of *Melicerta*, were only occasionally found in the first part, their population was very common in the wood distillation affected area.

Molluscs of which Gastropods, *Vivipara*, *Melania*, *Pleurocera* and *Indoplanorbis*, and two Pelyceopoda—*Lamellidens* and *Corbicula*—entirely disappeared from the affected region reappearing only gradually in Zone IV below showing their great sensitivity to pollution. Clusters of their colonies were seen in puddles formed of fresh sewage water far above the margin of the river water, the animals evidently having migrated to escape pollutional effects. Amongst the Entomostraca, one species of Copepoda, two of *Daphnia* and one of *Bosmina* only survived in the region. One of the former, a pink variety, was collected from the deep ooze in association with Chironomid larvae. Ostracods were not represented.

Of the aquatic Insects, Dytiscid and Hydrophyllid beetles, Nepidae (one species), Odonata—*Anisoptera* (Dragon fly nymphs), Culicidae, Chironomidae and Dixidae were recognized. *Eristalis* and Leptid fly larvae and pupae were rare in the mainly paper waste affected area above, but were collected in the second part below spots where wood distillation effluent mixed with the river water. An interesting black

Acridiid cricket, probably *Scelimena* sp., which was not observed in the clear waters above, was found in abundance in the margins hopping in and out of the water. This amphibious cricket (slightly smaller in size) was also collected in the sludge from depths of 7-9 ft. in scores during bottom hauls with the dredge. It was apparent that the species is associated with the septic sludge containing either a Bacterial sub-stratum or Tubificid worms (or both) as these may well form its food. A large number of Chironomid larvae, 'blood worms', was found in this region both from the margins and in muck in 8 ft. depth or more. Egg masses of this fly were obtained from the water margins. 'Water Scorpions', Nepidae, probably *Laccotrephes* (*Ranatra* was not found), were freely collected except at the vicinity of the outfall of the effluents. The insect is apparently unaffected as its breathing tube projects out of the water and it does not hence suffer oxygen depletion. Two species of Notonectidae and one of Corixidae were occasionally collected from many isolated areas, but their numbers were never high. Dragon fly nymphs were in abundance, their population greatly increasing towards the large pool below the road bridge. Mostly adult Dipteran insects, such as Midges, Chironomids, Snipe flies, Syrphus flies (Syrphidae), Dixa midges (Dixidae), Moth flies (Psychodidae) and Mosquitoes (Culicidae), were common along the affected river margin. Large numbers of Spiders and Ants (Hymenoptera) were found active along the damp margins, having obviously been attracted by the abundant food animals and decaying substances present. Many of these were also being carried by breeze into the running water thus accounting for their presence in fish guts.

A great increase in the occurrence of Bacillariophyceae and Myxophyceae, namely *Coscinodiscus*, *Pleurosigma*, *Navicula*, *Pinnularia*, *Gomphonema*, *Fragillaria* and *Asterionella*, was observed in this stretch. *Cyclotella* was first recorded here and showed an increase lower down. *Oscillatoria* and *Nostoc*, abundantly seen earlier, decreased relatively downwards. Of the Chlorophyceae only *Pandorina*, *Ankistrodesmus* and *Cosmarium* were represented in the river above the railway bridge, but the first two disappeared in the contaminated wood distillation waste part of the river.

(e) *Zone IV—Pool below Road Bridge.*—The main septic area is confined to a large pool some distance below the road bridge. This pool showed, at the time of these observations, all the characteristics of a heavily manured pond. There was a thick deposit of soft mud consisting apparently of humic matter and silt mixed with disintegrating pulp fibres which contributed towards an unprecedented growth of fauna and flora rarely seen in the course of a running stream. The hardly perceptible current further helped a more stabilized aquatic life to emerge and multiply. It is obvious from Table II that practically every group of aquatic organism is represented here unlike in a pond or a reservoir, where only a few groups can be obtained at any one time. The constant renewal of nutriment as well as an adequate supply of oxygen and carbon-di-oxide during the preceding months of stable water conditions, enhance chances for colonization of the pool by most varieties of organisms that may drift into it.

Free living Bacteria of types earlier mentioned were found abundantly in the sludge but the fungus colonies occurred only frequently. Amongst the Protozoans almost each type mentioned earlier together with several additional species, e.g. *Amoeba*, *Dydinium*, *Hydrosphaenia*, etc., were recorded. *Hydra* and Porifera (two species) reappeared in the pool. Turbellarians—*Catenula* sp. (?), free living Nematoda, etc., also increased particularly in the less polluted right portion of the river amongst vegetation. An abundance of Rotifers, as listed in the Table, gives a fair indication of the fertilizing qualities of the pollutants involved. Chaetopods, earlier listed, were less in density on account of mainly smaller shoaling fishes, which were found to feed upon them heavily.

Reappearance of Molluscan life in the pool is indicative of a certain measure of recovery, but some of the riverine forms of Gastropods, like *Pleurocera*, occurred

only along the infrequently seen clean sandy patches towards the comparatively sludge-free right margin. Gastropods were found to be more sensitive than Bivalves. Unionidae of which *Lamellidens* was collected from the sludgy depths of 5-6 ft. Many of them were the largest seen in any river, being about 4" in length. Several freshly dead Gastropods were also found and many had migrated to the exposed and half-submerged portions of rocks to avoid continued immersion in water as was evidenced by their clustering together. But on the right margin they were found amongst vegetation, where they also bred as seen by the mucilaginous masses containing their tiny shells. Though Leeches—Hirudinea—were unrepresented elsewhere, a species nearly $\frac{1}{2}$ " long was found in large numbers on one of the Gastropods, probably parasitic on it. A variety of Gastrotricha, as distinct from the one already noted in the polluted area earlier, was noticed amongst the vegetation. Entomostraca was the most abundant major animal group, swarming in the shallower regions of this pool, or creeping along the sub-stratum and vegetation, and some even found burrowing in soft ooze. A pink coloured Cladocera (the same as found in the pollutional area) up to a size of 4-5 mm. could be collected in abundance. Yet another species of a size well over 5 mm. was collected while half buried or creeping along the soft ooze, on the left polluted margin of the river. Brightly coloured Ostracods reappeared in this pool along with Hydracarinae (water mites) which were found in appreciable numbers. No crabs were noticed, but several prawns were found dead or dying, and a few well grown specimens were obtained on the vegetation.

Among insects, a species of Lepidoptera, probably a Pyralid or a Pyraustid larva, was found within cases built of *Hydrilla* leaves. These larvae showed 6-8 gills. Caddis flies (*Trichoptera*) were well represented, but Ephemerids and Plecopterans were rarely seen. Damsel fly nymphs reappeared in this area having been earlier recorded only in the clear water stretch. Dragon fly nymphs, however, were in great abundance. Flat bodied Naucoridae were gathered from 6-8 ft. depths in the dredge hauls particularly close to spots where two of the wood distillation waste channels emptied directly into the pool.

Amongst the aquatic vegetation, presence of numerous Desmids on the right side of the pool was a striking feature (Table II). Similarly, *Volvox*, *Pandorina*, *Eudorina*, *Euglena*, etc., reoccurred in this stretch. An abundance of *Pleurocera*, *Navicula*, *Pinnularia*, *Gomphonema*, *Fragillaria*, *Asterionella*, etc., amongst Diatoms was attributable to the pollutional effects as noticed previously, but their unabated intensity here was probably an indication of incomplete recovery. At the same time occurrence of *Spirogyra*, *Zygnema*, *Volvox*, *Hydrodictyon*, etc., showed that in parts, at least, some recovery had taken place. A variety of submerged vascular vegetation listed in the Table blanketed the pool contributing to the purificatory processes and also sheltered a rich aquatic life inclusive of fishes.

(f) *Zone V—Between the Pool and the Confluence.*—In this stretch commencing below the large pool for a length of 8 miles up to the river's confluence with the Thunga, the hydrological characteristics of clear water area above the weir were more or less repeated. The sub-stratum was sandy and the flow was comparatively fast interspersed with rapids and channels. Masses of black humic deposits with isolated fluffy 'Fungus' type Bacterial colonies of *Sphaerolitus* occurred occasionally. But numerous unicellular sulphur Bacteria were found in the soft ooze. Recovery cannot be said to have fully taken place even in this stretch as Tubificid worms were still seen, but their muddy holes were generally empty, the animals having been preyed upon by active minnows. Fauna and flora of this area, though quantitatively less than in the preceding area, was equally rich in variety. Additional organisms recorded here were *Chaetosphaeridium*, *Tribonema* and *Microsterias*. Sponges were much more in evidence especially near the confluence with the Thunga as in the clear waters. Nymphs of Caddis flies and stone flies were also collected.

V. EXPERIMENTAL OBSERVATIONS ON THE EFFECTS OF PAPER FACTORY AND WOOD DISTILLATION WASTES UPON FISH LIFE

Simultaneous with the foregoing biotal investigations, effects of various concentrations of effluents upon fish life were studied both by a few direct experiments and by extensive field observations. As elaborate methods for bioassays could not be undertaken, reliance was placed mostly on actual field data. As the main object was to estimate in broad terms the causes of damage to fish life directly by toxic effects and indirectly by hindering normal feeding, breeding and migratory habits of fishes—hence to fisheries, studies made are found fairly adequate.

(1) Experimental Studies on Survival of Fish

As dilution of waste ingredients in the river varies continually, it was hardly proper to use a sample of water taken at any particular spot to carry out survival tests in the laboratory. Assessment of percentage concentrations of toxic substances could not also be done owing to the complicated nature of mixtures and lack of facilities. Since, during casual field inspections, there seemed to be no obvious effort by fishes to avoid polluted waters, it was decided to carry out survival tests in different parts of the river itself by keeping some of the fish caught in submerged baskets and observing their survival for prolonged periods.

Four rectangular 'live' baskets of 20" × 14" × 12" dimensions with a small side door were made locally of thin bamboo strips so that any fish over 2½" in total length

TABLE III

Results of survival tests on fishes in differently effluent diluted points along the river course

Species of fish	No. of specimens	Clean water— Unpolluted	Paper wastes pollutional water	Mixed effluent pollutional water	Septic-cum- recovery pool
		Above Weir	Above Rly. Bridge	Between Rly. and Rd. Bridges	Below Rd. Bridge
		Point C Hours 4 8 24 48	Point P Hours 4 8 24 48	Point W Hours 4 8 24 48	Point R Hours 4 8 24 48
1 <i>Danio strigillifer</i> Myers and <i>D.</i> <i>malabaricus</i> (Jerdon) ..	3	3 3 3 3	1	3 3
2 <i>Mystucoleucus ogil- ibii</i> (Sykes) ..	2	2 2 2 2	2 2
3 <i>Chela argentea</i> Day and <i>C. clupeioides</i> (Bloch) ..	4	4 4 4 4	2 1	4 2
4 <i>Rasbora danico- nius</i> (Ham.) ..	4	3 3 3 3	4 3 1	4 4 3 2
5 <i>Esomus barbatus</i> (Jerdon) ..	2	2 2 2 2	2 1	2 1 1 1
6 <i>Cirrhitina fulungee</i> (Sykes) ..	2	2 2 2 2	2 2 1	2 1 1 1
7 <i>Garra jerdonsii</i> Day and <i>G. mullya</i> (Sykes) ..	3	3 3 3 3	2 2 2 2	3 3 2 2
Total ..	20	19 19 19 19	13 9 4 2	20 16 7 6

and about a thickness of $\frac{1}{2}$ " could not escape. Four points along the river were selected, approximately as shown in the sketch map: *C*—as control in the clear water, *P*—close above the railway bridge where paper effluents had been mixed with river water, *W*—between two bridges, where in addition to paper effluents, wood distillation wastes had got mixed about 120 yards above, and *R*—below the road bridge at the very end of the large pool where still an incomplete dilution of both effluents had occurred in the water. A number of small fishes of the region were obtained alive from the unaffected clear water above the weir. These belonged to seven different genera comprising *Mystocoleucus*, *Danio*, *Chela*, *Rasbora*, *Esomus*, *Garra* and *Cirrhitina* ranging in sizes approximately between 2½" and 6". In each basket 20 specimens as shown in Table III were introduced maintaining as nearly as possible a uniformity in size of different species. It is seen from the Table, that the selected species also can be broadly grouped into mainly surface or sub-surface, column and bottom feeding fishes. Except *Chela*, *Rasbora* and *Esomus* (which are found in stagnant waters also) the remaining species are mainly rapid water or hill stream forms. Survival of these fishes was noted every hour for the first four hours and later every four hours for a period of 48 hours continually.

Fishes of the stretch were obtained regularly by cast netting for a distance of 5 miles commencing from the clean water above the weir up to 2 miles below the septic-cum-recovery pool. It was later observed that the affected fishes were in the habit of concentrating in the latter pool for shelter and food not found elsewhere in such abundance in the stretch under study. By examination of these and the catches of local fishermen much valuable data were procured. All these fishes were examined for gut contents, signs of internal damages, as well as external marks of injury. Gut contents were noted, with the nature and volume of food. State of digestive organs, such as stomach, liver, etc., and any peculiarities exhibited in the blood vessels were recorded. Results of examination of 413 fishes are tabulated in the Appendix. Percentages of food have been calculated from averages of total in each species which includes one or two empty or full guts where 10 or more specimens were examined.

(2) Observations

When healthy *Rasbora*, *Chela* and *Danio* were introduced into the raw paper mill effluents in a glass jar, they died in 8–15 minutes exhibiting immediate distress. They showed violent gulping and jumping movements, flitted about in great confusion and died helplessly floating and recovering alternatively for short durations. All of them died keeping their *natural balance and not turning their belly upwards*. Death was later found to be due to severe asphyxiation and congestion of gills which had become swollen though there was no damage by bursting. Mucus on the gills was found to be coagulated even though body mucus seemed to have remained unaffected. In the raw wood distillation effluent, the same species of fish died within 2–3 minutes by still more violent gasping and jumping movements. They died dashing wildly against the sides of the jar *turning on their belly* with the operculum half closed and respiratory movements becoming feebler. Mucus on the body as well as on the gills was found to have coagulated and the lips and margins of the fin membranes had turned whitish. In samples of diluted river water taken between the bridges (where a certain amount of dilution by the wood distillation waste had taken place), these fishes died in 8–10 minutes. Death in this case was again due to asphyxiation of gills and loss of *equilibrium* as all these fishes floated with their belly upwards.

In experiments with fishes kept in cages it was noted that one fish had died in the clear water zone after 3 hours, evidently as a result of an earlier injury at the time of collection. Corresponding to the paper waste diluted water, though 13 fishes survived at the end of 4 hours at point *P*, by the end of 24 hours, 4 survived and only 2 at the end of 48 hours. At point *W*, no fish survived at all at the end of

4 hours. In the thoroughly mixed septic-cum-recovery pool at *R*, all 20 fishes survived for 4 hours, 16 at the end of 8 hours, 7 at 24 hours and 6 at the close of 48 hours.

The dead fishes were examined carefully. In many cases such post-mortem examination was rendered difficult as the dead fishes greatly putrefied within 4 hours. Most susceptible fishes in the order of their death were *Mystacoleucus*, *Chela*, *Danio*, *Rasbora*, *Esomus*, *Cirrhitina* and *Garra*. In fish which had died at points *P* and *R* the gills were not congested or choked by pulp, etc., but had turned whitish at point *W*. It looked as if paper effluents alone were responsible for death of fish at *R* (and also at *P*) but wood distillation waste was lethal at *W* being quick to act even at slightly lower dilutions. Later to these observations, it was found that no fish survived for more than 50–60 minutes in water samples taken between the bridges. Such lethal action apparently disappeared on higher dilutions below. All these dead fishes as well as the surviving ones showed empty guts indicating that they had either vomited or purged on coming in contact with the pollutants.

The surviving fishes in cages at *P* and *R* were in a dazed state as if narcotised. They were all swimming in a sluggish aimless manner, but none had lost equilibrium. There was a green tinge on the usually whitish coloured abdominal wall. None of these fishes attempted to escape when handled. This inactivity was not observed in fishes kept as control at *C* which were healthy and active even at the end of 48 hours. Majority of them having had no access to food showed almost empty guts. Further, in fishes at points *P* and *R*, the entire abdominal cavity was found to have turned yellowish and exuded a strong smell of chlorine and sulphides indicating thereby that the tissues were capable of absorbing these noxious gases. Choking of gills by pulp fibres or bacterial attacks was, however, not found to be the cause of death in any of these fish. On the other hand, the immersed cages were thickly covered by mucilaginous tufts of *Sphaerolitus* within 6–8 hours in all the three lower points.

VI. OBSERVATIONS ON FISH DISTRIBUTION AND CONDITION

In the course of investigations various species of fish found in the Bhadra at Bhadravathi were recorded and their relative scarcity, if any, noted. Fishes marked (*) are of economical value and those marked (ab) were either unrepresented or recorded very rarely from the affected stretch or above the weir in clear water.

- ab *Notopterus notopterus* (Pallas)
- Chela clupeoides* (Bloch)
- Chela argentea* Day
- * *Chela phulo* (Ham.)
- Laubuca atpar* (Ham.)
- ab *Barilius bendelisis* Ham.
- Barilius barila* Ham.
- Barilius barna* Ham.
- ab *Barilius gatensis* (Cuv. & Val.)
- ab *Barilius canarensis* (Jerdon)
- Danio strigillifer* Myers
- Danio malabaricus* (Jerdon)
- Brachydanio rerio* (Ham.)
- Esomus barbatus* (Jerdon)
- Rasbora daniconius* (Ham.)
- ab *Osteochilus nashii* (Day)
- ab *Osteochilus thomassi* (Day)
- Amblypharyngodon melittinus* (Cuv. & Val.)
- Aspidoparia morar* (Ham.)
- Barbus amphibius* (Cuv. & Val.)
- ab * *Barbus neilli* (Jerdon) = *Tor neilli* (Jerdon)
- ab * *Barbus khudree* (Sykes) = *Tor khudree* Sykes
- ab * *Barbus kolus* Sykes
- * *Barbus pulchellus* Day = *B. dobsonii* Day.

- ab * *Barbus filamentosus* Gunther = *B. mahecola* (Cuv. & Val.)
- * *Barbus sarana* (Ham.)
- Barbus sophore* (Ham.)
- Barbus dorsalis* (Jerdon)
- Barbus narayani* Hora
- * *Barbus lithopidos* Day
- Barbus chola* (Ham.)
- Oreichthys cosuatus* (Ham.)
- Cirrhitina fulungee* (Sykes)
- Garra jerdonii* Day
- * *Garra mullya* (Sykes)
- Garra bicornuta* Rao
- * *Labeo potail* (Sykes)
- * *Labeo dussumeiri* (Cuv. & Val.)
- * *Labeo porcellus* (Haeckel)
- ab * *Labeo fimbriatus* (Bloch)
- ab * *Labeo boggut* (Sykes)
- ab * *Labeo kawrus* (Sykes)
- ab * *Labeo ariza* (Bloch)
- * *Labeo calbasu* (Ham.)
- * *Schizmatorhynchus nukta* (Sykes)
- Rohitee neilli* Day
- Rohitee cotio* (Ham.)
- Mystacoleucus ogilbii* (Sykes)
- Lepidocephalichthys thermalis* (Cuv. & Val.)
- Nemachilichthys shimogensis* Rao
- Nemachilus striatus* Day
- Nemachilus bhimachari* Hora
- Nemachilus sinuatus* Day
- Nemachilus denisonii* Day
- Nemachilus anguilla* Annandale
- ab * *Ompok bimaculatus* (Bloch)
- ab * *Wallago attu* (Bl. & Schn.)
- ab * *Mystus seenghala* (Sykes)
- ab * *Mystus aor* (Ham.)
- Mystus vittatus* (Bloch)
- Mystus cavasius* (Ham.)
- ab * *Rita hastata* (Val.)
- ab *Rita pavimentata* (Val.)
- ab * *Bagarius bagarius* (Ham.)
- ab *Gagata itchkea* (Sykes)
- ab *Gagata viridescens* (Ham.)
- ab *Glyptothorax dekkaniensis* (Gunther)
- ab * *Pseudotropius taakree* (Sykes)
- ab *Pseudotropius atherinoides* (Bloch)
- * *Xenentodon cancila* (Ham.)
- Panchax lineatus* (Cuv. & Val.)
- ab *Ophicephalus gachua* (Ham.)
- ab * *Ophicephalus* spp.
- ab *Macropodus cupanus* (Cuv. & Val.)
- ab *Ambassis ranga* (Ham.)
- ab *Glossogobius giuris* (Ham.)
- ab * *Mastacembelus armatus* (Lacépède)
- ab *Macrogathus aculeata* (Ham.)
- ab * *Anguilla bengalensis* Gray

Of the above 79 species, nearly 30 are of economical value in the entire Thungabhadra drainage on account of the larger sizes attained. But of these 17 were seldom recorded in the affected river stretch, but were found mainly as stray young ones in the clear water above the weir. Well-known food fishes of the Thungabhadra, namely *Barbus neilli*, *B. khudree*, *Labeo fimbriatus*, *L. boggut*, *L. kawrus*, *L. ariza*, *Bagarius bagarius*, *Wallago attu*, *Mystus aor*, *M. seenghala*, *Rita hastata*, *Ophicephalus* spp. and a few others, were found only in one or two young specimens and so they can be considered as practically absent.

While engaged in these investigations, it was often noticed that local fishermen were making relatively rich hauls of fish down stream $\frac{1}{2}$ - $\frac{3}{4}$ mile from the paper mill.

effluent outfall. Though their catches were restricted (during April-May) to only a few medium or small-sized forms, those fish could have been of considerable market value elsewhere on account of the total bulk. But at the time, their value was only 15-25% of the value prevailing for the same fish if obtained from uncontaminated stretch. On examination, it was found that these fish had turned dirty yellowish in colour and smelled of paper waste sulphide and sulphonates. These odours were reported to persist even after the fish had been cooked.

On closer inspection of this polluted tract, a number of narcotised bottom-dwelling fish species *Nemachilus*, *Nemachilichthys* and *Garra* were picked from the shallow margins.* Several adult specimens of *Labeo potail* and *Barbus kolus* of lengths up to 21" were found floating dead or dying in an isolated pool cut off from the main flow of the river on the right margin (sketch map). These fish must have swam into this pool earlier on their own volition through an outlet as there was no inlet from the river. Death of fish in this region was obviously by slow degrees rather than by any immediate effect of the paper effluents. All these fish showed no external injury, but had a yellow pallor over the body, with eyes turned pale white. In none of them was there any evidence of choking of gills by pulp fibres, etc., as these gills were clean and pink in colour and the opercular movements, though normal, were rather slow. It was quite evident that the fish had become narcotised and gradually stupefied, by absorbing through the gills noxious gases and other chemical compounds dissolved in the water. This has been further confirmed during survival tests by an examination of dead or dying fish in cages which showed not only a similar bad odour but also a yellow pallor of the body.

That these fish easily become prey to various piscivorous birds was noted at the time. There was a good deal of activity especially in the early hours of the morning when the birds used to pick the aimlessly floating or swimming fishes. Droppings of these birds showing fish bones were found along the margins commonly. Many crows and cranes were also busy on the margins picking up any fish that chanced to come to the edge or surface of the water.

An examination of digestive organs of several specimens (Appendix) indicated mainly empty stomachs and intestines; but the blood vessels lining the digestive tracts and the adjoining body musculature within the abdominal cavity appeared congested and swollen, many showing pustules or suppurating spots. In several others, liver had become discoloured and pulpy to touch when a microscopic examination revealed that fatty degeneration had set in. A strong mixed odour of sulphides, sulphonates and chlorine emanated on opening these fishes. Even from the apparently healthy fish collected by the author and purchased from the fishermen, such odours were detected. These observations coincide with the state of the surviving fishes in the cages as already narrated. It may be of interest to mention that Westfall and Ellis (1944) have also noticed that in fishes subjected to pulp mill pollution there is an undesirable flavour of the flesh by absorption of unpleasantly flavoured substances both from the liquor effluents and from decomposition products of disintegrating pulp.

The active 'sight' feeders, *Rasbora*, *Esomus*, *Chela* and *Danio*, were found darting from the right, effluent-free clear water portion of the river course into the effluent diluted left side (in the region below the railway bridge) and dart back again within a few seconds. This peculiar habit was found on close examination to be due to the presence of dense colonies of Tubificid worms, Chironomid larvae, etc., in the polluted portion, on which organisms the fish freely used to feed. Such to and fro movements were repeated many times and usually shoals of these minnows could be found in the clear water portion engaged in sorties for feeding in the polluted portion. It is significant that the above fishes and a few *Barbus pulchellus*, *B. lithopidos*,

* Similar instances of stupefied fish occurring have also been noticed in Bihar where the paper mills of Dalmianagar discharge the effluents into the Sone river.

Cirrhina fulungee and *Garra* spp. were also found to be somewhat hardier than the rest of the fish observed. Though all these ultimately succumb to the injurious effects of the pollutants, the unaffected portion of the stream seemed to afford a certain degree of safe retreat; what percentage of such affected fish finally recover is not known. It was also noted that the bottom feeding fishes showed, more commonly, the typical pathological conditions than the above-mentioned column feeders. In the available species of *Labeo*, *Schizmatorhynchus*, *Osteochilus*, *Barbus kolus*, *Garra*, *Nemachilus* and *Nemachilichthys*, all of which are more or less mainly bottom feeders, pathological conditions were observed much more frequently than in others.

VII. GENERAL DISCUSSION

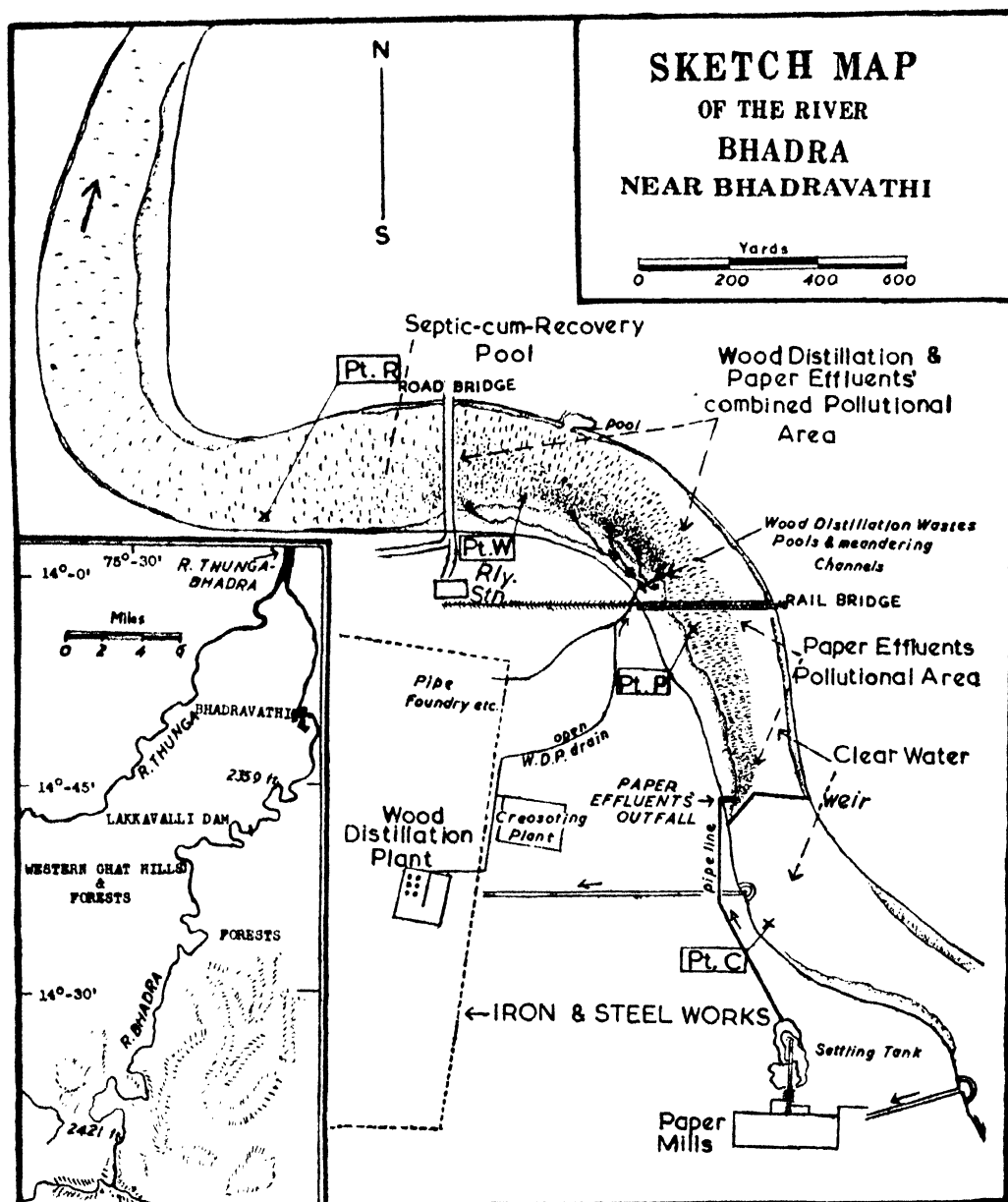
(a) Direct Effects

From the data given in the foregoing chapters and the preliminary field observations, only broad conclusions can be drawn as to the possible manner in which the pollutants affect the Bhadra river fisheries.

Alkalinity of up to 9 is known to be tolerated by fully developed fresh-water fishes and none of the industrial alkalies like NaOH have clearly been shown to be lethal to fish (Doudoroff and Katz, 1950). This may explain the survival of many fish in the paper effluents diluted water for longer periods. As there is a sufficiently large amount of CO₂, i.e. as much as 5.48 p.p.m. in the river water and any large increase in pH is counteracted by it, excess of alkalinity may be safely eliminated as a major cause of fish mortality.

Presence of small quantities of hydrogen sulphide and free chlorine are well known to be toxic to fish to an extreme degree. Usually 1.00 p.p.m. and, under certain circumstances, even as low as 0.1 p.p.m. of these compounds have been stated to be lethal to fish. Doudoroff and Katz (*op. cit.*) have concluded that even traces of these substances in polluted waters should be regarded as a possible hazard to fish life. In the Bhadra, values of total sulphides varied between a maximum of 15.2 p.p.m. to a minimum of 2.04 p.p.m. depending on the distance from the outfall, and in the lower stretch it was still as much as 0.82 p.p.m. Similarly dissolved chlorine content varied between 19.00 and 7.56 p.p.m. within the affected stretch. That both these values are very high during the critical summer months when there is a greatly attenuated stream flow for prolonged periods and are far in excess of considered safe minimums of 1.00 p.p.m. cannot be overlooked. Theoretically, though effluents should dilute nearly 200 times, accumulation of pollutant substances in water and along the sub-stratum can easily take place in the highly broken up river bed in its numerous channels and bayouts. As sensitivity of fishes to these substances varies from species to species, absence of several forms in the 12 miles stretch is not surprising, since they may easily avoid sulphide diluted waters as observed by Carpenter (1930). During experiments on the toxicity of sulphurous acids fish died due to coagulation of mucus on gills causing asphyxiation. Shelford (1917) has reported that 16–19 p.p.m. of sulphur-di-oxide gas dissolved in the tap water was enough to kill small fishes in one hour. But 0.5–1.00 p.p.m. of hydrogen sulphide and dissolved sulphides are stated to be critical concentrations for more sensitive fishes under average water quality conditions. Total sulphide values noticed (15.2–2.04 p.p.m.) in the affected stretch are quite high and, even 9 miles below, as much as 0.82 p.p.m. were traceable. These are decidedly lethal dosages for fish however favourable other conditions may be. In the absence of experimental evidence, it is to be presumed that the high 'sulphur' bacterial activity noticed helps in a great measure of recovery of the water and very likely the accumulated sludge, when taken as or along with food by the bottom feeding fish, may release sulphur or its compounds on digestion which may finally affect the fish. Decomposition of lignin sulphonates and pulp may also produce various substances which also could act as specific poisons. Under prolonged exposure to dissolved toxic substances, the fish appear to get firstly

narcotised, finally succumbing to the lethal effects as seen by field collections. Depletion of oxygen and a marked degree of anaerobicity do not seem to be causes of fish mortality as indicated by the few observations on the distribution of organisms and field tests.



Sketch map showing the Bhadra river stretch at Bhadravathi indicating points of observations, demarcation of effluent diluted areas, disposition of outfalls, etc. The entire stretch, except the area of the septic-cum-recovery pool close below the road bridge, is rocky and cut up into numerous channels which however are not shown.

In the wood distillation effluent area, death of fish is almost instantaneous. Adam, Potter and Murdoch (1937) have found that even non-toxic tar washings had irritant effects on fish. During experiments they noticed that phenols of 10.00

p.p.m. in water caused twitching, loss of equilibrium and partial narcosis. Other tar bases as low as 2.00 p.p.m. caused loss of equilibrium in $2\frac{1}{4}$ hours. 1.00 p.p.m. of acridine (a substance known to be present in the distillation washings of wood as well) caused violent gulping and narcosis with loss of equilibrium in 48 minutes. Erichson Jones (1951) found that solutions of phenol, ortho- and para-cresol are highly toxic to minnows, and that they appear to have some specific poisonous effect on the nervous system of fish causing them to lose their sense of balance and capability of co-ordinated movements. He concluded that minnows show little or no capacity for recognizing and avoiding the solution at any concentration between 0.04% and 0.0003% of solution and become intoxicated; he also mentioned that 0.004% is about the threshold of toxicity for phenol. The effect at higher concentration is neuro-muscular in nature.

Presence of sulphuric acid from the steel plant in the wood distillation wastes, is perhaps only slightly toxic to fish, since 10.00 p.p.m. of free acid causes grave danger to fish as noted by Doudoroff and Katz (*op. cit.*). Coagulation of gill mucus is usually caused by acids, but this cannot be attributed entirely to sulphuric acid, as there are several other organic acids in solution in the wood distillation effluents.

In general it is evident that the causes of death of fish in effluents from wood distillation plant are more toxic to fish populations at higher concentrations and on dilution their effects apparently disappear. But in the paper mill effluents, though the toxic effects are slow to manifest, continued exposure of fish to even dilute concentrations has a narcotic effect which ultimately becomes fatal.

(b) Indirect Effects

Presence of pathogenic conditions in fish reveals that continued exposure to the effluent diluted waters affects their physiological functions, causing degeneration of liver, congestion of blood vessels and appearance of suppurative spots by interfering with blood circulation. Whether these conditions are due to 'acidimic', i.e. toxic effects acting internally, is difficult to ascertain in the absence of well-defined experimental data. But most species of fish that appear to be unable to distinguish toxicity in lower concentrations are attracted into the Bhadra from the Thungabhadra below. The chemical attraction exerted by the pollutants in low dosages and the presence of increased food organisms seem to impel several species to move up to the pollutional area. According to Stephen (1936) fish cannot distinguish certain chemical wastes as such, resulting in their ultimate stupefaction and mortality. In fact, they show preference to certain chemical wastes, as some varieties are attracted and others repelled. However, once the fishes enter the Bhadra, they may be unable to resist the slow combined narcotic effects of the ingredients in both effluents and may even become intoxicated. Ingestion of humic and septic sludge containing varieties of sulphur bacteria and accumulated sulphur compounds may release complex substances within the body, and ultimately cause death of such fishes. An accumulation of sulphides, ligno-sulphonate and sulphite smells inside fish obtained occasionally from waters comparatively less smelling of these compounds 5-6 miles below is otherwise difficult to explain. Stoppages of blood in smaller capillaries and gills may well be caused by relatively minute doses of these substances. By osmosis through gills some of these dissolved gases can be transmitted directly to other parts of the body, causing 'stasis' or stoppage of blood circulation within the capillaries, abdominal wall and intestine, etc. These gases too may have slow anaesthetic effects upon fish as already noted.

In gravid examples of *Barbus kolus*, *Labeo dussumieri*, *L. potail*, *L. calbasu*, etc., the smell of sulphur compounds was much more intensive than in immature ones. In all these cases the gonads were coloured dirty yellow as against the usual healthy white or pale pink colours. There seemed to be a tendency on the part of the mature fish to store the compounds more easily within the body tissues than in young ones.

Such fish are definitely rendered incapable of spawning as their gonads are impaired if the fish itself is not destroyed earlier. Recovery of such fish at any rate seems impossible under normal conditions. The lower rate of breeding and incapacitation of all adults that wander into the Bhadra must tell heavily upon the total fish population of the entire Bhadra system if not the Thungabhadra, as most fish seek breeding grounds at the headwaters during monsoon months.

Eggs and larvae of monsoon breeding fishes that migrate from far down the river may escape destruction during the rainy part of the year for about 4 months (between middle of June and October). But as a result of harm to the gonads, no fish that enters the affected region when the gonads mature during the pre-monsoon periods is free from permanent injury. Hence there is a progressive destruction of upward migrating fish population at the important pre-monsoon season. Further, adults of several fish may not rise into the Bhadra at all from the Thungabhadra, due to slight chemical pollution noticeable at the confluence between the two rivers. Otherwise it is difficult to explain the almost total absence of important fishes like *Labeo fimbriatus*, *Barbus kolus*, *B. neilli*, *Tor khudree*, *Mystus aor*, *M. seenghala*, etc., even though they form the most important items of food fishes in the Thunga barely 10 miles away under identical hydrological conditions. Though due to the monsoon floods all signs of septic sludge and other substances in the river course are annually removed, at least in the case of pre-monsoon breeders and the post-monsoon spawning *Tor* sp. (Mahseers), their spawning grounds are sure to be affected below Bhadravathi for a distance of 11-12 miles. Since the production capacity of the mill is nearly doubled after these studies, it is to be expected that owing to the discharge of more effluents, the conditions in this section of the river have further deteriorated from the fisheries point of view.

VIII. SUGGESTIONS

As a result of these investigations the following tentative suggestions can be offered to safeguard fish life in the river as much as practicable.

(1) Possibilities of recovering digester washings for re-use on a larger scale than has been done hitherto may be explored thus reducing the quantity of water used by improved washings of pulp. Chlorine, sulphonates, sulphides, sulphurous acid and such toxical agents may be reduced or at least rendered innocuous by suitable means. If the total quantity of water is reduced, perhaps it may be possible to store the effluents in 'lagoons' for annual washings into the swollen river when floods occur.

(2) As sulphur content is high in the washings contributing to a rich growth of sulphur bacteria, which indirectly affects fish, its reduction may be sought. Perhaps use of manganese oxide instead of calcium oxide as a base for digester cooking may be useful.

(3) Stagnation of raw liquor for lengthy periods may not give required results as the chemical contents inhibit bacterial activity so necessary for natural recovery. As nitrogen is hardly present in quantity in the paper wastes, mixing them with domestic sewage to start bacterial action may be a possible line of work.

(4) As sulphate pulp wastes from bamboo are rich in sugars, manufacture of by-products like ethyl alcohol or fodder yeast may be tried. Small quantities of anilin, oxalic acid, adhesives or even fertilizers may perhaps be successfully manufactured from other substances.

(5) Percolation of the effluents through river sand may be tried, thereby removing the toxic wastes. The debris accumulated may be mechanically removed and may even be re-used after purification.

(6) As far as possible cellulose pulp and lignin should be removed by efficient means as these resistant plant matters begin to disintegrate in water only after a long time.

(7) Steps have to be taken to safeguard the stretch which may gradually become enriched towards the base of the Lakkavalli Dam (a few miles above) by an accumulating fish population trapped between the dam and effluent-ridden zone. Being the head-waters of the Thungabhadra, it is likely that many fish spawn in the Bhadra waters during monsoon months and this has to be investigated further.

IX. SUMMARY

The ecological features of a section of the Bhadra river affected by waste effluent discharges of a pulp and paper mill and a wood distillation plant are examined near Bhadravathi, Mysore State. Fish distribution and their survival in variously affected parts of the river are discussed. A list of organisms is given and the peculiarities of their distribution mentioned. Some chemical and analytical results of water are also discussed in relation to fish life.

(1) It was noticed that majority of well-known food fishes numbering about 18 out of about 36 in the river were almost absent in the affected stretch.

(2) The few adult fish found during April-May in the intensely affected two miles stretch and many smaller forms showed pathogenic conditions, such as narcosis, 'statis' of blood capillaries, fatty degeneration of liver, etc. They also possessed an unsavoury flavour which reduced their market value.

(3) During survival tests,

(a) *In paper mill effluents*: fish died by asphyxiation brought about by a coagulation of gill mucus presumably due to the presence of free chlorine, sulphides, sulphates, sulphurous acid and other potent sulphur compounds as well as strong alkaline mixtures. At higher dilutions, gradual narcotical effects seemed to set in.

(b) *In wood distillation wastes*: at higher concentrations fish died by shock and coagulation of mucus on the body and gills. Gills were also found swollen, showing that severe asphyxiation had taken place. At higher dilutions, such symptoms apparently disappeared.

(4) Mortality of fish was not so much due to deoxygenation of surface waters as much as to the toxicity contained in it. Soft sub-stratum, however, was highly anaerobic with a thick blanket of characteristic 'fungi'. But a thorough deoxygenation of the region was not observed during the period of study. Periodic large-scale mortality of fishes was nevertheless reported when uncoverable digester washings were let out.

(5) Certain varieties of fishes avoided the polluted stretch almost completely, and a few were, however, attracted either by the 'chemical' attraction or more so by the increased amount of food organisms which ultimately proved fatal. This danger was greater when almost ripe spawners tried to ascend during the minor floods of May-June when pre-monsoon showers occurred.

(6) An increased organic life was noticed due evidently to sugars and other organic substances (pulp-cellulose) and their components released on disintegration. Such biological activity was inhibited higher up by the sterilizing action of the chemical substances such as SO_2 , Cl_2 , etc., contained in the wastes, but was pronounced when waters became diluted. Lack of nitrogen in the compounds also retarded bacterial activity in the earlier stages.

(7) The stretch has become a barrier for all migrating fishes, particularly to those that migrate upwards during pre-monsoon months and downwards during post-monsoon months. As the rocky and sandy stretch below for 8-9 miles gets covered over by septic sludge with bacterial colonies, the pre-monsoon spawners, like *Myxus aor*, *M. seenghala*, and probably *Bagarius bagarius* and the post-monsoon or winter spawners like *Tor khudree* (Mahseer), etc., do not find congenial breeding grounds.

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* These references were not consulted in original.

(Several references consulted for identification of various biotal organisms and fish are not mentioned.)

Showing number of fishes actually netted, nature of pathogenic symptoms and gut

Sl. No.	Genera or species of fish	Clear water— Zone 1	Unpolluted right part of the river— between outfall and road bridge	Paper waste polluted portion—between outfall and railway bridge
1	2	3	4	5
1	<i>Aplocheilichthys lineatus</i> (Cuv. and Val.) ..	4—0	2—0	..
2	<i>Chela</i> spp. ..	32—20 30–50% full, Entomostraca, may fly and damselfly nymphs.	24—15 40–50% full, pulp fibres, insect egg masses, ants and Dytiscid beetle remains.	2—2 Empty guts. <i>Liver turned pulpy.</i>
3	<i>Danio</i> spp. ..	24—15 30–40% full, Entomostraca, Rotifera, Hydracarinae, insect remains.	13—13 25–40% full, Tubificidae, Chironomidae, insect remains.	..
4	<i>Barilius</i> spp. ..	5—5
5	<i>Labeo utpar</i> (Ham.) ..	22—5 10–20% full, insect remains.	8—5 Empty, traces of ants and beetles.	..
6	<i>Rohita</i> spp. ..	20—10 30–40% full, Hydracarinae, Dytiscid beetles.	1—1 30% full, insect remains.	..
7	<i>Mystacoleucus ogilbyi</i> (Sykes) ..	24—0	8—0	..
8	<i>Rasbora daniconius</i> (Ham.) ..	30—10 25–40% full, Entomostracans, Hydracarinae, Chironomids, and insect remains.	22—15 25–60% full, Tubificidae, Chironomids, <i>Oscillatoria</i> , <i>Nostoc</i> , <i>Ulothrix</i> and Diatoms.	5—5 Empty.
9	<i>Ambassis rangi</i> (Ham.) ..	16—0	..	1—0
10	<i>Barbus ticto</i> (Ham.)	16—0
11	<i>Barbus amphibius</i> (Cuv. and Val.) ..	12—5 5–10% full, Protozoans, Entomostraca, Hydracarinae, and Diatoms.	2—2 Empty.	..

APPENDIX

contents. (Bold numerals indicate number caught, small numerals, those examined.)

Combined paper and wood distillation affected part—between bridges. Zone III	Septic-cum-recovery pool. Zone IV	Recovery stretch up to confluence. Zone V	Remarks
6	7	8	9
..	Very rare.
15—10 25-35% full, Tubificid worms, <i>Spirogyra</i> , <i>Oscillatoria</i> , <i>Nostor</i> and sponge spicules.	16—10 Almost empty, ant remains, Rotifers, Entomostracans, dead wood.	4—4 80-90% full, insect remains (gorged) and Entomostracn.	.. Several specimens were picked up dead in affected stretch.
24—15 Generally empty. Traces of Hemiptera, Coleoptera, Rotifers, <i>Ulothrix</i> , <i>Pediastrum</i>	These do not occur in pollutant mixed waters.
4—4 25% full, Tubificid worms, <i>Eristalis</i> maggots, <i>Spirogyra</i> , vascular plant remains. Picked also dying in the stretch.
..	Very rare.
..	Very rare.
..	Not found in affected stretch.
35—15 10-30% full, Tubificids, Chironomids, insects, <i>Spirogyra</i> , <i>Ulothrix</i> , Diatoms.	8—8 25-35% full, Chironomids, insect remains and Entomostraca.	17—10 10% full Tubificids, Entomostracans, insect remains.	.. Seems to be a hardy fish, thriving in the region.
..
5—5 Almost empty. Traces of insect remains and algae.	Appears to be rare in affected waters.
..	..	2—2 30% full, <i>Paramaecium</i> , Ostracods, Chironomids, <i>Oscillatoria</i> and sand.	2—2

APPENDIX

Showing number of fishes actually netted, nature of pathogenic symptoms and gut

Sl. No.	Genera or species of fish	Clear water Zone I	Unpolluted right part of the river—between outfall and road bridge	Paper waste polluted portion—between outfall and railway bridge
1	2	3	4	5
12	<i>Barbus narayani</i> Hora ..	14—0
13	<i>Barbus sophore</i> (Ham.) ..	20—0
14	<i>Barbus sarana</i> (Ham.) ..	8—5 25-40% full, Entomostracans, Hydraeae, insect remains and sand.	2—2 Empty. Yellowish tinged body, smelly inside.	..
15	<i>Barbus neilli</i> (Jordon) ..	13—6
16	<i>Barbus pulchellus</i> Day ..	4—4 30-45% full, Coleoptera, Desmids, Spirogyra and sand.
17	<i>Barbus kolus</i> Sykes	2—2 Empty
18	<i>Barbus lithopidos</i> Day ..	4—4 40-60% full, Dytiscids, Chironomids, Copepods, Diatoms, Desmids and sand.	9—9 40-75% full, pulp fibres, bacterial tufts, Protozoans, insects, <i>Spirogyra</i> , and <i>Hydrilla</i> .	5—5 Almost empty, traces of sludge, Diatoms, and vascular plants.
19	<i>Osteochilus thomasi</i> (Day) ..	15—10 20-60% full, Entomostraca, Chironomids, ooze and sand with Protozoans.	3—3 Empty. Abdominal musculature yellowish.	..
20	<i>Cirrhitina fulungee</i> (Sykes) ..	12—10 Sand, <i>Pandorina</i> , Desmids and Diatoms.	4—4 10% full, mud, sponge spicules, Diatoms.	..

—contd.

contents. (Bold numerals indicate number caught, small numerals, those examined.)

Combined paper and wood distillation affected part—between bridges. Zone III	Septic-cum-recovery pool. Zone IV	Recovery stretch up to confluence. Zone V	Remarks
6	7	8	9
..	Rare in the river stretch affected but abundant above weir.
..
1—1 Empty	1—1 Empty	..	Pathogenic specimens, found stupefied.
..	..	1—1 10% full, insect remains, Tubificid, Desmids, <i>Spirogyra</i> and <i>Hydrilla</i> .	Only juveniles seen above the weir.
..	3—3 35% full, Coleopterans, Entomostracans, sand.
1—1 Almost empty, traces of vascular plants, insects, and sludge.	.. 80–90% full, Tubificids, pulp fibres, Protozoa, Diatoms, Desmids and <i>Oscillatoria</i> .	3—3 15–25% full, insects, Tubificids, <i>Hydrilla</i> and <i>Spirogyra</i> .	Juveniles only.
5—5 Almost empty, traces of bottom sludge, bacterial tufts, Diatoms, Desmids.
12—10 50–60% full, bottom sludge, bacteria, Rotifers, Diatoms.	5—5 60–80% full, ooze, bacteria, Diatoms and Desmids.	3—3 70% full, sludge, bacteria, <i>Arcella</i> , <i>Diffugia</i> and other Protozoans.	Pathogenic specimens.
18—10 Mostly empty, sponge spicules, pulp fibres, traces of Cladocerans, <i>Spirogyra</i> , and Desmids.	This fish is mainly a bottom feeder.

APPENDIX

Showing number of fishes actually netted, nature of pathogenic symptoms and gut

Sl. No.	Genera or species of fish	Clear water— Zone I	Unpolluted right part of the river— between outfall and road bridge	Paper waste polluted portion—between outfall and railway bridge
1	2	3	4	5
21	<i>Esonus barbatus</i> (Jerdon) ..	15—10 40-60% full, Ento- mostracans, Chiro- nomids, mud and sand with Proto- zoans. (Sarcodina)	3—3 Empty. Yellowish musculature.	
22	<i>Labeo calbasu</i> (Ham.)	..	1—1 Empty. Abdomen yellowish with odour of sulphides.	2—2 50-60% full, sludge with bacteria, Pro- tozoans, Diatoms, pulp fibres, Nema- tode worms.
23	<i>Labeo potail</i> (Sykes)	3—3 40-50% full, sand, Diatoms and Des- mids, traces of Protozoans.	2—2 70-80% full, sludge, sponge spicules, Desmids, vascular plant remains.	2—2 Empty. Yellowish; congestion of blood vessels, suppurative spots, odour present.
24	<i>Labeo porcellus</i> (Haeckel)	1—1 Only traces of sludge and Nematodes (Pa- rasitic ?).
25	<i>Labeo dussumieri</i> (Cuv. and Val.)
26	<i>Schismatorhynchus</i> <i>nukta</i> (Sykes)	..	1—1 Empty. Yellowish tint in abdominal musculature.	2—2 Empty. Suppura- tive spots on intes- tines, liver pulpy.
27	<i>Garra</i> spp. ..	42—10 35-90% full, <i>Spiro-</i> <i>gyra</i> , Desmids, Dia- toms.	10—5 15-60% full, sand and mud, <i>Spiro-</i> <i>gyra</i> , Desmids and Diatoms.	..
28	<i>Nemachilus</i> spp. ..	15—10

—contd.

contents. (*Bold numerals indicate number caught, small numerals, those examined.*)

Combined paper and wood distillation affected part—between bridges. Zone III	Septic-cum-recovery pool. Zone IV	Recovery stretch up to confluence. Zone V	Remarks
6	7	8	9
<p>12—10 30-60% full, bottom ooze with Sarcodina, sulphur bacteria, Rotifers, Diatoms and Desmids.</p> <p>..</p>	<p>5—5 70-80% full, ooze with bacterial threads, Diatoms, Desmids.</p>	<p>8—8 60-80% full, sludge, bacteria, Chironomids, Protozoans, Diatoms.</p>	..
..	<p>3—3 Empty. <i>Yellowish; intestines with congested blood vessels and suppurative spots. Smell of sulphides.</i></p>	..	Adult specimens show high degree of pathogenicity.
<p>3—3 Empty. <i>Yellowish; congestion of blood vessels with suppurative spots and odour.</i></p>	<p>3—3 60-75% full, sludge. Infusorians, Rotifers, Chironomids, pulp, bacterial tufts, early signs of yellow tint.</p>	<p>1—1 25% full, sand, sludge, bacteria, pulp fibres, sponge spicules.</p>	do
..	Very rare.
<p>3—3 40-60% full, sludge, bacterial threads, Protozoans, Diatoms and pulp fibres.</p>	Stray adults only examined.
<p>1—1 Almost empty. Sludge, pulp fibres, Diatoms, Desmids.</p>	<p>1—1 60% full, sludge, Sarcodina, bacterial threads, Diatoms, <i>Oscillatoria</i>.</p>	..	Pathogenic adults.
<p>9—5 30-40% full, sludge, bacteria, <i>Oscillatoria</i>, <i>Nostoc</i>.</p>	<p>6—5 Empty, traces of pulp fibres, <i>Oscillatoria</i> and Diatoms.</p>	<p>6—5 80-90% full, sludge, bacteria, Diatoms, Desmids.</p>	..
..	<p>5—5 Traces of sludge, pulp, Diatoms.</p>	..	Often found dying.

APPENDIX

Showing number of fishes actually netted, nature of pathogenic symptoms and gut

SL No.	Genera of species of fish	Clear water. Zone I	Unpolluted right part of the river— between outfall and road bridge.	Paper waste polluted portion—between outfall and railway bridge
1	2	3	4	5
29	<i>Xenentodon cancila</i> (Ham.)	1—1 One whole <i>Rasbora</i> .	..
30	<i>Mastacembelus armatus</i> (Lacépède)	1—1 Empty, full of Trematode worms, probably parasitic.
31	<i>Mystus</i> spp. ..	6—0	1—1	..
32	<i>Ompok bimaculatus</i> (Bloch)

—concd.

contents. (*Bold numerals indicate number caught, small numerals, those examined.*)

Combined paper and wood distillation affected part—between bridges. Zone III	Septic-cum-recovery pool. Zone IV	Recovery stretch up to confluence. Zone V	Remarks.
6	7	8	9
..
3-3 Empty. Blood vessels congested, specimens turned yellow.	Pathogenic symptoms— lethargic adult speci- mens.
..
..	1—1 Empty.

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STUDIES ON THE NEUROSECRETORY SYSTEM OF *IPHITA* *LIMBATA* STÅL. (PYRRHOCORIDAE: HEMIPTERA)

PART IV. OBSERVATIONS ON THE STRUCTURE AND FUNCTIONS OF THE CORPORA CARDIACA OF THE ADULT INSECT

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CONTENTS

	<i>Page</i>
1. Introduction	171
2. Material and methods	172
3. Observations	
(a) Endocrine system of <i>Iphita</i>	173
(b) Neurosecretory connections	174
(c) Structure of the corpus cardiacum	174
(d) Experimental investigations	
(i) Myotropic activity	176
(ii) Chromatophorotropic activity	178
4. Discussion	180
5. Summary	183
6. Acknowledgements	183
7. References	183

1. INTRODUCTION

The corpora cardiaca were originally described by various authors as lateral sympathetic ganglia, pharyngeal ganglia, corporis faringei, etc., regarding them as parts of the sympathetic system of the insect. Ontogenetically, they may be considered as ganglia because they develop as invaginations of the stomodaeum in the neighbourhood of the hypocerebral ganglion and later move to the wall of the aorta to which they become intimately connected (*vide* Hanström, 1939).

The structure of the corpora cardiaca has been studied in detail only by a few authors. Pflügfelder (1937) has pointed out that the corpora cardiaca consist of nervous and osmiophil cells and he has attributed a secretory or excretory function to the latter. Casal (1948) has made a comprehensive and comparative study of the retrocerebral organs of insects, and in his monograph he has described briefly the corpora cardiaca of *Pyrrhocoris apterus*. A more detailed account of the structure of the corpus cardiacum of *Locusta migratoria* has been given by the author (Nayar, 1954).

While considerable work has been done on the physiology of the corpus allatum, only a few workers have attempted to study the functional significance of the corpora cardiaca. Evidences have recently accumulated to suggest an endocrine function for these structures (*vide* Scharrer, 1955). Recent researches on the neurosecretory system of insects by Scharrer (1952) have shown that the corpora cardiaca function as storage organs for the neurosecretory products elaborated by the special cells of the *pars intercerebralis* of the brain of *Leucophaea maderae*. A similar observation was made by E. Thomsen (1952) in *Calliphora* and subsequently by various authors in several groups of insects. E. and B. Scharrer (1944) and Hanström (1953) have drawn a comparison between the hypothalamo-hypophyseal

system of vertebrates and the intercerebralis-cardiacum-allatum system of insects. E. Thomsen (1952) has shown that the corpus cardiacum of *Calliphora* can replace the neurosecretory cells in inducing the development of the eggs. Bodenstein (1953) in his work on *Periplaneta* suggested the existence of a specific interaction of the corpora cardiaca with the prothoracic gland causing the maintenance and activity of the latter. He showed that removal of the corpora allata and the retention of the corpora cardiaca would help to maintain the prothoracic glands in the adult insect. But Wigglesworth (1955), in his study of the breakdown of the thoracic glands in adult *Rhodnius*, showed that transplantation of the corpus cardiacum will not help to prevent the breakdown of the glands. Vannucci (1953) has shown that extracts from corpora cardiaca contain a heart-activating substance which may be either adrenalin itself or a closely related substance. A myotropic activity has been recorded in the extracts by Cameron (1953) and Koller (1954).

That the corpora cardiaca of insects have a chromatophorotropic activity on crustacean pigment cells has been reported by Brown and Meglitsch (1940) and M. Thomsen (1943).

A detailed investigation on the neurosecretory system of the common plant bug *Iphita limbata* Stål. (Pyrrhocoridae: Hemiptera) was started in this laboratory in 1953. A comprehensive account of the neurosecretory cells of the nerve ring and some aspects of their enzyme content have been published by the author (Nayar, 1955a, b). Accounts of the structure of the corpus allatum and the neurosecretory pathways have also been published (Nayar, 1956a, b). The present paper embodies the results of observations on the structure and some functions of the corpus cardiacum of *Iphita*.

2. MATERIAL AND METHODS

Adults of both sexes of *Iphita* were collected from the field and kept in insectary jars or boxes where they thrived on cotton seeds soaked in water or sap of *Plumaria*.

The pronotum and the dorsum of the head were removed and the insect was pinned flat in a dish of insect-Ringer. Under the stereoscopic binocular ($\times 40$) the brain with the median trachea supplying the pars intercerebralis could easily be located. Lying close to it and placed posteriorly is the anterior end of aorta. Lateral to the aorta could be seen the tiny, opaque, whitish corpora cardiaca, which could be easily removed together with the corpus allatum when the trachea is dissected away.

The following methods were used in this investigation:

1. Freshly dissected glands were gently flattened in a drop of insect-Ringer on a slide for examination under the phase-contrast and dark-ground microscopes. The spheroids were studied in living cells by staining in 0.001% neutral red.

2. For general observations, material fixed in Bouin's, Helly's or Orth's fluid was sectioned and stained in Heidenhain's iron haematoxylin.

3. For neurosecretory connections, the entire nerve ring together with the aorta, the anterior end of the gut and the endocrine glands were fixed in Bouin's or Smith's fluid, and sections were stained in Gomori's chrome alum-haematoxylin-phloxine (Gomori, 1941).

4. For lipochondria, Aoyama's, Kolatchew's and Baker's sudan black (Baker, 1949) methods.

5. For phospholipids, Baker's (1946) acid haematein and pyridine extraction tests.

6. For diphenols, Lison's chromaffine tests (Lison, 1953), indole reaction (Pearse, 1953), Gibb's method for argentaffin granules (Gomori, 1953), Giemsa method for adrenochrome after Sevki (Pearse, 1953), Vulpian reaction, acid diazonium reaction for aromatic amines and phenols (Pearse, 1953), hexamine-silver method of Gomori (1953), and Hillarp and Hökfelt (1955) method for noradrenalin and adrenalin.

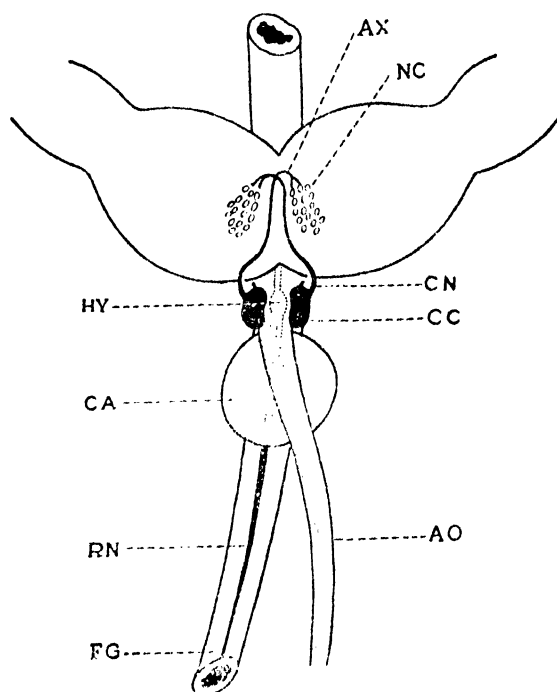
7. Chromatographic methods for diphenols (Block, Durrum and Zweig, 1955) after Roux (1951) and Barton, Evans and Gardner (1952).

8. For experimental investigations on myotropic activity, isolated hindgut of *Iphita* (Pyrrhocoridae) and *Aspongopus janus* (Fabr.) (Pentatomidae) were used. For studying chromatophorotropic activity, *Caridina laevis* Heller (Decapoda) among the Crustacea, *Aplocheilus lineatum* (Cuv. and Val.), *Barbus vittatus* (Day), and *Etiopius maculatus* (Bloch) among the fishes, and the lizard *Hemidactylus brookii* Gray were used as test animals.

3. OBSERVATIONS

(a) The endocrine system of *Iphita* (Text-fig. 1)

Situated in the pars intercerebralis of the brain of *Iphita limbata* Stål., is a pair of clusters of neurosecretory cells. Each cluster is composed of sixteen cells and the two clusters lie just underneath the membranous investment of the brain below the level of the longitudinal trachea. Springing from each cluster is a nerve composed of the axons of the neurosecretory cells, which run forwards and down-



TEXT-FIG. 1. Diagram of the endocrine system of *Iphita*. AO—aorta; AX—axonic pathway from neurosecretory cells; CA—corpus allatum; CC—corpus cardiacum; CN—cardiac nerve; FG—foregut; HY—hypocerebral ganglion; NC—neurosecretory cells of the brain; RN—recurrent nerve.

wards, and after forming a chiasma run posteriorly. At the posterior phase of the brain, each emerges out as the cardiac nerve running to the corpus cardiacum. The two corpora cardiaca are tiny glands, each measuring about 182 to 195 μ in diameter, lying on the two sides of the anterior end of the aorta and intimately connected to its walls. They extend to the ventral edges of the aorta where they meet and fuse forming a bridge-like mass (Plate XIX, C). Lying between the lobes of the corpora cardiaca and shifted posteriorly is the median hypocerebral ganglion. A large corpus allatum lies attached to the ventral wall of the aorta, just behind the

hypocerebral ganglion. In the adult male this gland measures about 390μ in diameter, in the young adult female it is 350μ in diameter, while in the gravid female it is 910μ in diameter. The corpus allatum is connected to the corpora cardiaca by a median allatic nerve.

(b) *Neurosecretory connections*

A detailed account of the neurosecretory connections to the retrocerebral endocrine organs of *Iphita* has been given by the author (Nayar, 1956b). When stained in Gomori's chrome alum-haematoxylin-phloxine, the pathways along which the neurosecretory matter from the cells of the pars intercerebralis gets transported could easily be made out in the form of blue granules and irregular lumps of colloids on a red phloxinophilic background. The axons from the neurosecretory cells of the brain serve to transport the colloids, stainable blue, to the corpora cardiaca, where they aggregate as clumps and granular masses among the cells and along the edge of the gland. Through the allatic nerve the neurosecretory colloids pass into the corpus allatum. At the junction of the allatic nerve, the recurrent nerve of the stomatogastric system also is in contact, and through this region neurosecretory materials flow into this sympathetic cord also (Text-fig. 1). Actual flow of neurosecretory material into the blood by percolation through the aortic wall also has been observed in *Iphita*.

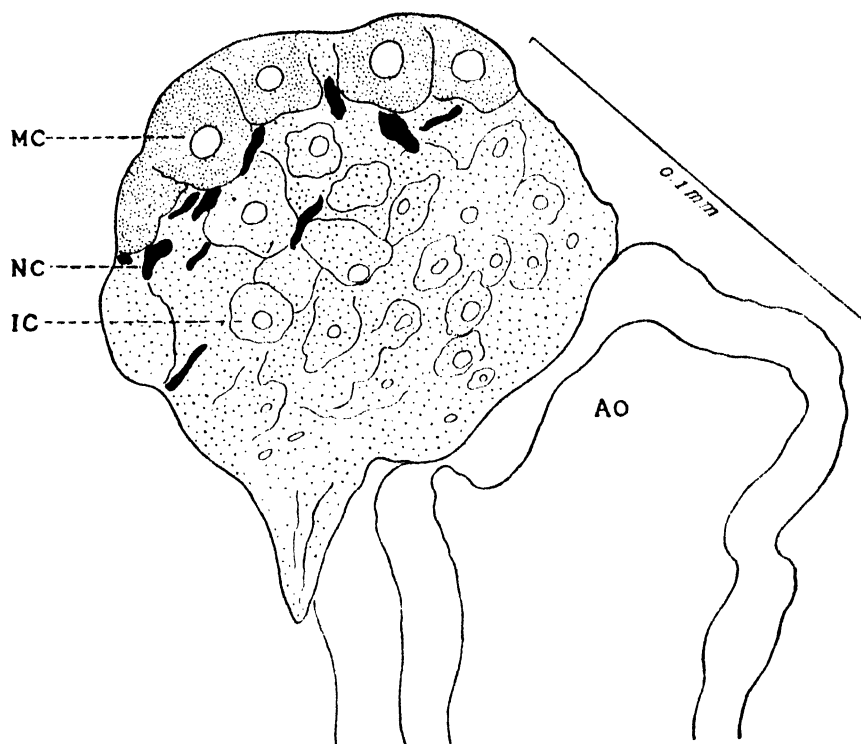
(c) *Structure of the corpus cardiacum*

Under the stereoscopic binocular, the corpora cardiaca appear as white, opaque bodies. In this respect they resemble the neurosecretory cells of the brain. Like the neurosecretory cells, the corpora cardiaca also show up as shiny, bluish-white structures under dark ground illumination (Plate XIX, A). When the preparation is very gently pressed, the extremely thin, membranous envelope of the gland breaks and colloids and granules flow out which remarkably resemble the shiny bluish-white products of the neurosecretory cells. The cells of the corpora cardiaca remain intact and shiny, and it is evident that the shiny matter that flows out of the gland represents the contents outside these cells, suggesting that they may be the neurosecretory matter. This assumption is supported by the finding that the corpora cardiaca of insects, where the neurosecretory cells have been extirpated three days earlier, show only faint, bluish-white matter flowing out or remaining inside the gland. The gland in this case as a whole loses the whitish colour and appears pale brownish.

Under the phase-contrast microscope the corpus cardiacum is seen to have a set of large cells which could be easily dislodged by slight pressure. These cells have rounded nuclei with dark chromonemata and conspicuous chromocenters (Plate XIX, B). They measure from 13 to 20μ in diameter with nuclei of about 10.1μ in diameter. Their cytoplasm shows a granular appearance with the granules often clumped together to form irregular masses. Inner to this layer of cells could be seen the fibres of the cardiac nerve as streaks, which show on them spheroids. Close to these streaks could be seen aggregates of spheroids with clear interior and dark rims, representing the neurosecretory matter accumulating in them. The rest of the gland shows a cytoplasm which is granular and which does not present a homogeneous nature. Here often the cells appear elongated when the preparation is well pressed, measuring about 15 to 20μ in length with a nucleus of about 8μ in diameter situated in the broader part of the cells.

When stained in 0.001% neutral red, the cells show vesicular and transparent nuclei and among the large cells and along the track of the axonic bundles could be seen large spheroids which take up the red colour. These spheroids measure from 0.48 to 1.43μ in diameter, the majority being about 0.71 to 1.08μ in diameter. These neutral red spheroids are similar to those of the neurosecretory cells.

In routine sections stained with iron haematoxylin after fixation in Orth's or Bouin's fluid, the peripheral larger cells could be seen in a well-preserved condition (Plate XIX, C). They show well-defined nuclei and granular cytoplasm, the latter taking up a blue stain in Gomori's chrome alum-haematoxylin. Among these cells and lying inner to this cellular tier are granular clumps, which generally stain bright blue in chrome haematoxylin (Text-fig. 2; Plate XIX, D). However, in some

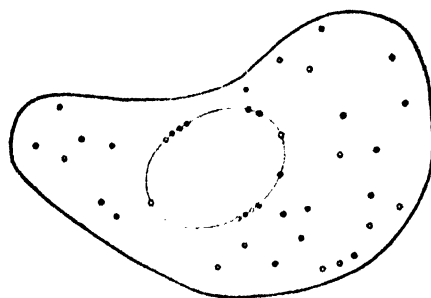


TEXT-FIG. 2. Camera lucida drawing of a section through the corpus cardiacum of *Iphita*, showing colloids in intercellular regions and among the nerve fibres. The peripheral cells with granular matter also shown. AO—aorta; IC—inner cells; MC—peripheral cells; NC—neurosecretory colloids among cells.

preparations these colloids take up a shiny red colour thus appearing phloxinophilic; the true nature of this coloration is not understood. This is the neurosecretory matter collected in the gland. The smaller nuclei in the rest of the gland take up the red component of Gomori's stain.

The spheroidal bodies seen in the axonic region of the corpus cardiacum represent the lipocondria as evidenced by staining in sudan black by Baker's method. They produce the characteristic blackened pictures in the classical 'Golgi preparations' like Aoyama's and Kolatchew's methods. They are thus sudanophil, argentophil and osmiophil.

The granular substances of the cytoplasm of the larger cells are to a certain extent argentophil as they reduce silver in Aoyama's method (Text-fig. 3). In osmium methods no satisfactory pictures have been obtained. The black granules produced by Aoyama's method form only a part of the granular constituents of these cells. They are seen as scattered bodies on the outer wall of the nuclear membrane also. In sudan black-staining such granules are feebly coloured greyish-black; they are poorly coloured in neutral red also.



0.01 mm

TEXT-FIG. 3. Camera lucida drawing of a peripheral cell of *Iphita* in an Aoyama preparation, showing spheroids reducing silver. Such spheroids are seen in the cytoplasm and along the nuclear membrane.

When the whole gland is treated with 1% osmic acid, the large cells become brownish and remain like that for about a day. Soon the whole gland becomes brownish-black and the details are lost. From this it could be inferred that these larger cells form the osmiophil cells of the gland. It could also be concluded that the granular-spheroidal contents of these cells constitute a substance different from the neurosecretory matter though they are coloured blue in chrome alum-haematoxylin.

The phospholipid content of the corpus cardiacum, as evidenced by Baker's acid haematein test and confirmed by pyridine extraction tests, is seen as blue concretions and granular masses in the region of the neutral red-positive spheroidal structures of the lipochondria. They are abundant along and near the axonic tracts and among the large cells. The cellular regions of the gland are only poorly coloured. The contents of the neurosecretory cells of the brain are rich in phospholipids, and identical matter is seen clumped in the axonic regions and adjacent portions of the corpora cardiaca.

The chromaffin content of the corpora cardiaca has been studied by various authors with conflicting results. In *Iphita*, when tests were made on whole glands according to Lison's method, faint yellowish coloration was seen. No granular material was seen giving positive reactions. A faint violetish colour was developed in certain cases in indole reaction tests when sections were incubated for sixty minutes at 60°C. But negative results were obtained when gland sections were treated with diazotised *p*-nitroaniline, hexamine-silver and weak ferric chloride. No special staining effect was noticeable when material fixed in Orth's fluid was treated with dilute Giemsa according to Sevki's method.

Chromatograms run at room temperature with butanol : acetic : water (4 : 1 : 5) on Whatman No. 1 paper by circular or descending methods, when sprayed with ferric chloride/potassium ferricyanide (Block, Durrum and Zweig, 1955), gave no positive blue coloration at all. Extracts of ten corpora cardiaca in one ml. of ethanol or distilled water were used for spots, each of 0.02 ml. volume. Carbonic acid : water at pH 4.2 (Barton *et al.*, 1952) was also used as a solvent. Spraying with weak ferric chloride or sucrose-HCl-ethanol (Roux, 1951) also gave no indication of any phenolic substances on the chromatograms.

(d) *Experimental investigations*

(i) *Myotropic activity*.—Cameron (1953), Wigglesworth (1954, citing Cameron) and Koller (1954) have drawn attention to the myotropic activity of the extracts of the insectan corpus cardiacum. The hindgut of *Iphita* when isolated in insect-Ringer showed no definite peristalsis. Some sporadic contractions were noticed,

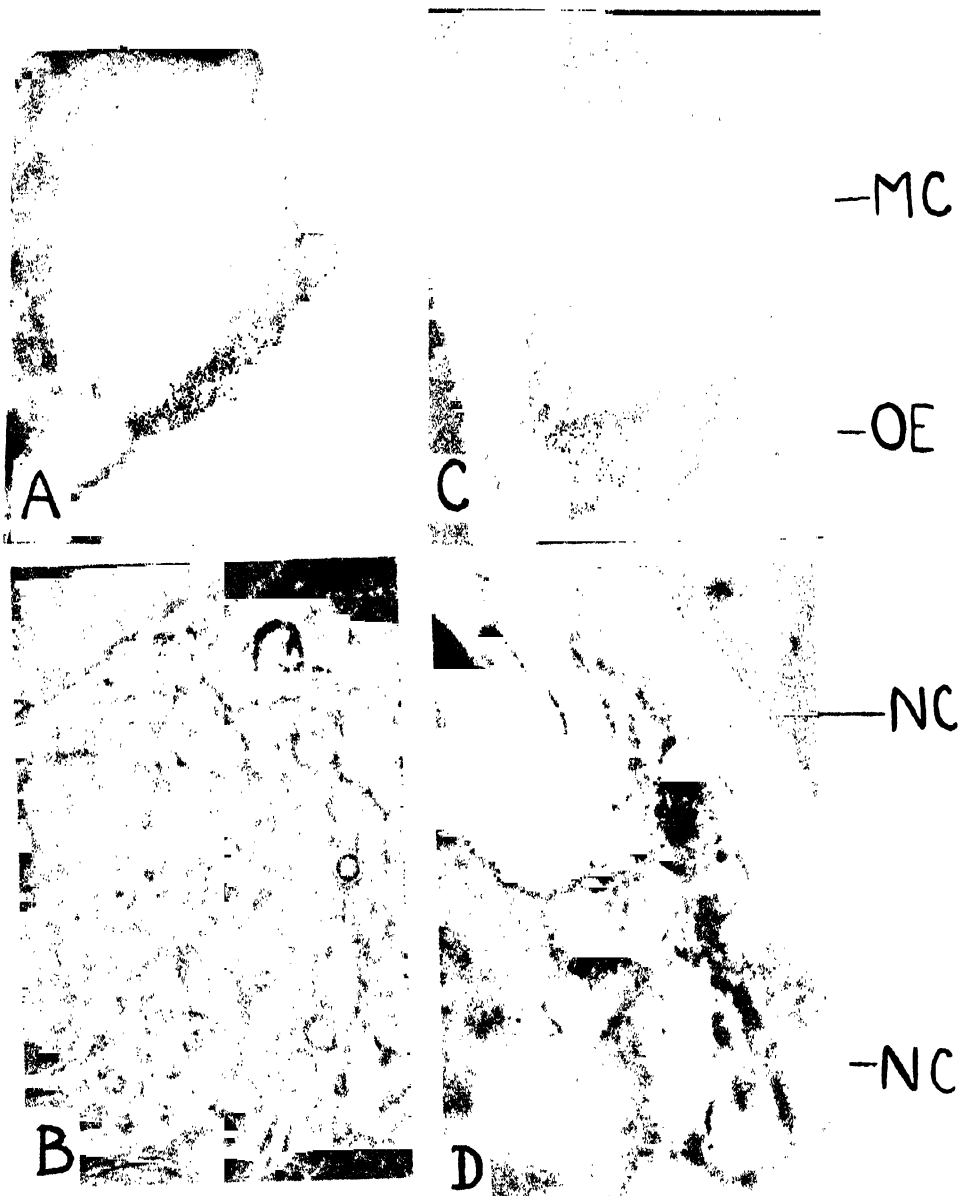


FIG. A. The corpus cardiacum under dark-ground. The shiny whitish mass of the droplets and cells, released by the rupture of the gland, resemble the neurosecretory cells and their products. Approx. $\times 170$.

FIG. B. The cells of the corpus cardiacum under phase contrast. A few cells are seen in the centre of the figure which are the marginal cells. Approx. $\times 480$.

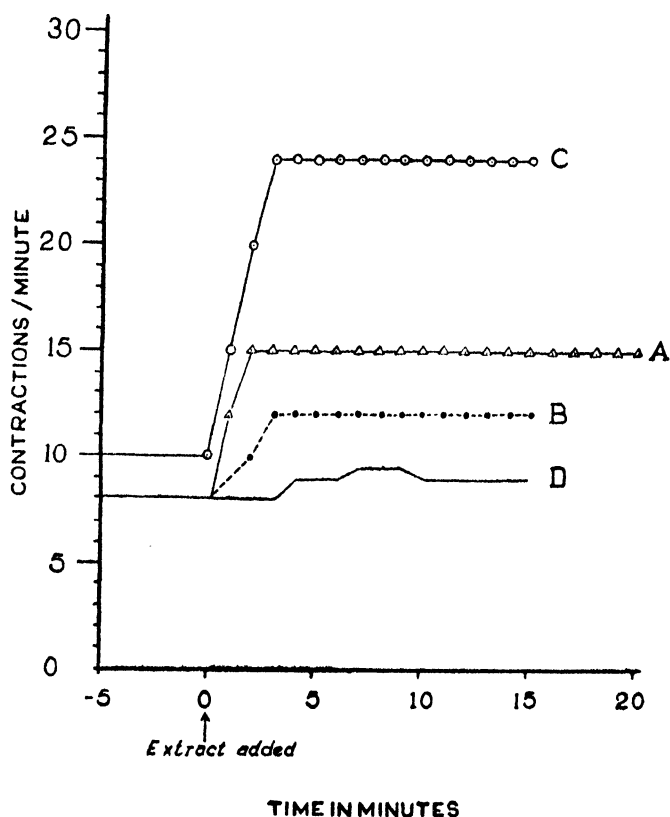
FIG. C. Section showing the peripheral large cells (MC) of the corpora cardiaca distinctly. OE = oesophagus. Fixation Orth. Stained in Heidenhain's iron haematoxylin. Approx. $\times 120$.

FIG. D. Section of the corpus cardiacum, showing accumulation of neurosecretory colloids (NC) among cells and along the margin. Fixation Bouin. Stain Gomori's. Approx. $\times 1,200$.

but it was found that these could not be advantageously studied in experiments. Examining the hindgut of another bug *Aspongopus janus* (Fabr.), (Pentatomidae: Hemiptera) it was seen that when isolated and cleaned from debris, it would continue to exhibit peristalsis in insect-Ringer for more than ninety minutes. The rate of peristalsis starts at about 10 per minute, which in a few minutes will fall to 8 per minute. It will continue to show regular contractions at this rate for more than an hour at room temperature (86° to 88°F.).

Five pairs of corpora cardiaca of *Iphita* were crushed and added to 1 ml. of Ringer and mixed well. After centrifugation at 5,000 r.p.m. for ten minutes, the supernatant was used as the extract.

A preparation of the hindgut of *Aspongopus* in a dish with 5 ml. of Ringer was used for the experiments. When 0.2 ml. of the extract was added to the Ringer, no noticeable effect was seen, but when 0.5 ml. of the extract was added, the rate of peristalsis increased, becoming 10 per minute in two minutes and rising up to a rate of 12 per minute in three minutes. After fifteen minutes it fell to 10 per minute at which rate it continued for more than thirty minutes (Text-fig. 4).



TEXT-FIG. 4. The rate of contractions per minute of the isolated hindgut of *Aspongopus*. A—when extract of corpus cardiacum of *Aspongopus* was added; B—when extract of corpus cardiacum of *Iphita* was added; C—with isolated hindgut in Holtfreter solution of pH 6.6 with extract of corpus cardiacum of *Iphita* added; D—with extract of corpus cardiacum of *Iphita* deprived of neuro-secretory cells added.

When the corpora cardiaca of *Aspongopus* itself were used, it was seen that the frequency of peristalsis rose from 8 to 15 per minute at which rate it continued for over thirty minutes (Text-fig. 4).

It is worth mentioning here that granular bodies coloured brownish-yellow were seen in the cytoplasm of the cells of the corpora cardiaca of *Aspongopus* when the glands were treated with 10% potassium iodate followed by formalin according to Hillarp and Hökfelt's (1955) method. Such a granular structure was not seen when the glands of *Iphita* were treated likewise.

The pH of the Ringer, in which the preparation of the hindgut was placed for experiments, had been seen to have a rôle in deciding the frequency of peristalsis. The pH of stock insect-Ringer used for the study ranged from 3.5 to 3.7, while fresh Ringer had a pH of 4.6. Freshly mixed Holtfreter solution giving a pH of 6.6 was used as a medium, and it was observed that the hindgut of *Aspongopus* contracted 10 times per minute for a considerable time. When extracts of corpora cardiaca were added the rate of peristalsis rose to 15 per minute in one minute and reached up to 24 per minute in three minutes' time. It continued to contract at this rate for a long time. The variation of the rate of contractions of the hindgut at different pH is given in Table I.

TABLE I

Table showing the rate of contractions per minute of the isolated hindgut of *Aspongopus janus* in physiological solutions of different pH

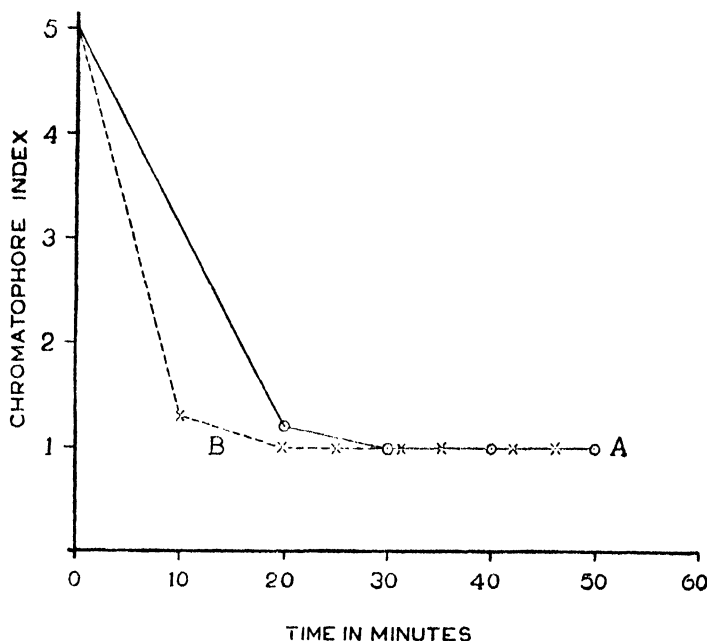
pH of Ringer	Average normal contractions/minute	Average number of contractions/minute on addition of extract
3.5	8	12
3.7	8	12
4.6	8	12
6.6	10	24
7.2	8	15 (irregular)
8.0	nil	nil

Neurosecretory cells from brain either extracted or directly added to the medium produced no increase in the rate of peristalsis. Corpora cardiaca from insects deprived of their neurosecretory cells three days earlier showed definitely a rise in the rate of peristalsis bringing it to 9 to 10 per minute.

(ii) *Chromatophorotropic activity*.—Corpora cardiaca of insects have been stated to possess a chromatophorotropic activity (Brown and Meglitsch, 1940; M. Thomsen, 1943). The common freshwater shrimp *Caridina laevis* was selected as a test animal. Here the animal has red chromatophores which completely disperse (reticulate type) converting it into a dark animal in dark surroundings and which concentrate (punctate type) rendering it pale in clear and sandy background. Hogben and Slome group the chromatophore changes in animals into five steps (chromatophore index 1 to 5), viz. punctate, punctate-stellate, stellate, stellate-reticulate and reticulate (Parker, 1948), to designate the degrees of dispersal or concentration of pigments. In *Caridina*, reticulate measures about 170 μ in diameter, stellate-reticulate about 130 μ , stellate about 104 μ , punctate-stellate about 52 μ , and punctate about 13 to 20 μ . These represent the stages 5, 4, 3, 2 and 1 in Text-fig. 5.

In the shrimps where eyes are removed, the red chromatophores disperse completely to stage 5 irrespective of the environment and they failed to concentrate as the regulatory eye-stalk hormones were wanting. In such animals entire corpus cardiacum or extracts of ten glands in 1 ml. of distilled water made as suggested by M. Thomsen (1943) were administered. When the corpus cardiacum was injected wholly by a fine glass needle into the body of an eyeless shrimp, the animal reacted

in a few minutes by concentrating the red chromatophores. In about 10 minutes' time they became punctate-stellate and in 20 minutes' time they concentrated fully to stage 1. All the red chromatophores did not show the same effect, a few contracted slowly. 0.5 ml. of extracts injected into the body gave the same results; 0.2 ml. of the extract concentrated the chromatophore comparatively slowly. Adding extracts to bits of branchiostegites removed and kept in insect-Ringer also gave the same results, the rate of contraction being somewhat slower (Text-fig. 5).



TEXT-FIG. 5. The chromatophore index of *Caridina* under the influence of the extract of the corpus cardiacum of *Iphita*. A—tests on isolated branchiostegites; B—results of injection of extracts into eyeless shrimps.

The neurosecretory cells showed no effect on the pigment cells of *Caridina*.

Though the corpus cardiacum can induce concentration of the red chromatophores in Crustacea, their effects on vertebrate pigment cells have not been studied so far.

Isolated scales of fishes belonging to *Aplocheilichthys lineatus* and *Barbus vittatus* put in distilled water showed their melanophores somewhat dispersed. The extracts of corpora cardiaca or crushed corpora cardiaca placed on these scales produced no effect on the melanophores. In *Etroplus*, the isolated scale removed to distilled water shows a few melanophores punctate and a few somewhat dispersed. The corpus cardiacum has no effect on these melanophores, but the neurosecretory cells of the pars intercerebralis of the brain when crushed and placed on the scale were seen to induce a dispersal of some of the punctate melanophores. The change was slow and extended over 30 minutes.

Hypophysectomized lizard *Hemidactylus brookii* was also used as a test animal. After hypophysectomy the animal turns pale, and blanching is completed in about 40 minutes. Within about a week the animal begins to shed its skin in large patches.

Three pairs of corpora cardiaca of *Iphita* were crushed into a paste and put into the thigh of such hypophysectomized lizard. This produced a local expansion of the chromatophores. They began to expand in about 20 minutes and the

dispersal lasted for about 150 minutes. The action was localized in the regions of the thigh and the pelvis.

In another experiment, freshly dissected corpora cardiaca (a pair) of *Iphita* were pushed with a fine glass needle into the thigh of a light-adapted pale animal. The chromatophores of the region began to disperse in about 15 minutes.

The neurosecretory cells of the insect showed no effect on the lacertilian chromatophores.

4. DISCUSSION

The general distribution of the endocrine glands in *Iphita limbata* resembles that of *Pyrrhocoris apterus* described by Cazal (1948).

The corpus cardiacum of *Iphita* is not syncytial and in this respect it differs from that of *Locusta* (Nayar, 1954) and the corpus allatum of *Iphita* itself (Nayar, 1956a). The distinction of chromophobe and chromophil cells could be made out in *Iphita* also, as has been noticed in other insects by Cazal (1948). The chromophil cells are also osmiophil as observed by Pflügfelder (1937). But a well-defined osmiophil nature could not be seen in *Iphita* beyond an initial stage as osmic acid is reduced by the entire gland.

The neurosecretory connections of the corpora cardiaca of *Iphita* have been discussed fully by the author (Nayar, 1956b). The corpus cardiacum receives a good amount of the neurosecretory material which gets collected as colloids among the cells and on the axons. This material, stainable blue by chrome alum-haematoxylin, accumulating in the corpora cardiaca has been observed by other authors also like Scharrer (1952) in *Leucophaea*, E. Thomsen (1952) in *Calliphora*, Arvy, Bounhiol and Gabe (1953) in *Bombyx*, Arvy and Gabe (1953) in *Odonata* and mayflies, Arvy and Gabe (1954) in *Plecoptera* and M. Thomsen (1954) in *Hymenoptera*. Curiously enough in some preparations it has been seen that these concretions in the corpora cardiaca of *Iphita* become phloxinophil. The true nature of this tintorial reaction could not be made out. Scharrer (1955) cites examples where neurosecretory matter in insects stains red in Gomori's chrome alum-haematoxylin-phloxine; this staining property applies to the melanophore-expanding hormones of the vertebrates also.

The use of dark-field microscopy in the study of living neurosecretory cells and their products, together with phase-contrast examinations, have become increasingly popular (E. Thomsen, 1954; Nayar, 1955a). The presence and distribution of the neurosecretory content which is refractile could be made out clearly by these methods.

An additional proof of neurosecretory material accumulating in the corpus cardiacum has been afforded by the acid haematein tests performed in the present studies. The phospholipid content of the neurosecretory cells and their axons could be seen in the axonic tracts of the cardiac nerve and the regions around it and near and among the cells of the cardiacum.

The granular mass of the cytoplasm of the cells of the corpus cardiacum represents the secretion of the gland itself. Though this also is stainable blue by chrome alum-haematoxylin, it does not resemble the neurosecretory matter because it reacts poorly to lipochondrial techniques and it does not show a rich phospholipid content. In *Plecoptera*, Arvy and Gabe (1954) have shown that the cellular contents of the corpora cardiaca stain red in Gomori's stain.

Koller (1948) observed that extracts from the head of a number of insects would bring about an acceleration of contractions of the malpighian tubes. Working from that point, Cameron (1953) pointed out that extracts of the corpora cardiaca of *Periplaneta* will increase the activity of the malpighian tubules of *Locusta* and will accelerate the rate of contraction of the hindgut of *Locusta* and *Periplaneta*. The same extracts were shown also to increase the rate of heart beat of *Periplaneta* by 50 per cent (*vide* Wigglesworth, 1954).

Koller (1954) showed that the extracts of the corpus cardiacum of *Carausius* would retard the contractions of the gut of beetles, while extracts of the corpus allatum and intestines would accelerate it.

In the light of these conflicting findings, it is interesting to note that the rate of contractions of the hindgut of *Aspongopus* gets accelerated when the extracts of the corpora cardiaca are added to the medium, thus confirming Cameron's observations. Extracts of corpora cardiaca of *Iphita* exerted a comparatively feeble acceleration.

The volume of a pair of corpora cardiaca of *Iphita* is approximately 0.006 mm.³ In an extract of five pairs of glands in 1 ml. the strength may be about 1:3-40,000. When 0.2 ml. of the extract was added to the gut preparation in 1 ml. of Ringer, no noticeable effect was observed, but when 0.5 ml. of the extract was added the rate of peristalsis showed an acceleration. This shows that a dilution of 1:200,000 was ineffective, while 1 in about 80,000 was effective. It suggests that probably the content of the active principle in the corpus cardiacum of *Iphita* is lesser than that of *Periplaneta* (Wigglesworth, 1954).

The significance of pH in inducing increased rate of contraction has been noted by Koller (1954). Here, in *Iphita* also the maximum contractions have been observed around pH 6.6.

The facts that the neurosecretory cells and their extracts do not accelerate the contractions of the gut, and that corpus cardiacum of an insect deprived of its neurosecretory cells earlier could induce this acceleration, suggest that the myotropic activity is due to substances in the corpus cardiacum and not essentially due to neurosecretory matter. This substantiates similar conclusions drawn by Cameron (1953).

Brown and Meglitsch (1940) and M. Thomsen (1943) have recorded the chromatophorotropic activity of the insectan corpora cardiaca. Both have used Decapod Crustacea as their test animals. The chromatophorotropic activity of the corpora cardiaca of *Iphita* on the shrimp *Caridina* is similar to that observed by the above workers on their experimental animals. The active principle of the corpus cardiacum brings about a contraction of the expanded red chromatophores of *Caridina*. Functionally this principle is thus similar to the eye-stalk-hormone of Decapoda.

The influence of invertebrate hormones on the colour change of vertebrates has been studied by some authors (*vide* Hanström, 1939). The results obtained by the various investigators have been conflicting. Abramowitz (1936) states that eye-stalk extracts of *Palemonetes* expanded the melanophores of hypophysectomized *Mustelus*, *Rana*, and *Anolis* and dispersed the chromatophores of the fish *Chrosomus* and hypophysectomized *Amieurus*. The effect of insectan corpus cardiacum, which physiologically shows a chromatophorotropic activity similar to that of the eye-stalk-hormone of the crustaceans, has not so far been tested on vertebrates. In the present investigation it has been shown that generally the piscine melanophores do not react to the extracts of corpora cardiaca, but the latter brings about a dispersal of pigments in the melanophores of light-adapted and hypophysectomized *Hemidactylus*.

Hormones have been demonstrated to bring about concentration or dispersal of chromatophores in vertebrates. The chief element that brings about the dispersal is intermedine or the 'B' hormone of the pituitary (Parker, 1948). Adrenalin has been seen to bring about blanching. By hypophysectomy the lizard is denied the intermedine necessary for the dispersal of pigments and this fact has been observed in *Hemidactylus brookii* by Noble and Bradley (1933), who have also studied the process of moulting in the animal accompanying the removal of the pituitary. A supply of intermedine artificially will bring about dispersal of the chromatophores in such animals. The present study shows that a similar but

localized dispersal of pigment could be induced by supplying the active principles of the corpus cardiacum also.

Though the melanophores of fish scales do not react to the corpora cardiaca, some of the concentrated pigment cells of the scales of *Etroplus* show a dispersal when crushed neurosecretory cells of the brain are placed on them.

It is interesting to note that the corpus cardiacum of *Iphita* possesses chromatophorotropic activity which is of no use to the animal. This insect is not capable of any kind of colour change and this endowment of a chromatophorotropic principle may be the attribute of the hormone which produces myotropic and other effects in the animal. In some insects like *Corethra*, however, the corpus cardiacum in common with the brain secretes a substance related to adrenalin causing dispersal of melanophores over the air sacs (DuPont-Raåbe, 1949).

The chromatophorotropic activity is not impaired by the removal of the neurosecretory cells earlier from the insect.

The nature of the active principle or principles of the corpus cardiacum has been worked out to some extent by Cameron (1953). Workers like DuPont-Raåbe (1949) and Vannucci (1953) have suggested that adrenalin or adrenalin-like substances are elaborated by these glands.

The chromaffine test as elaborated by Lison (Lison, 1953) has been positive on the whole glands. Using larvae of *Tenebrio*, Cameron (*vide* Wigglesworth, 1954) showed three spots in chromatograms, of which one lies between Rf 0.3 and 0.4, which when eluted from the paper revealed the same pharmacological effects as the gland-extract itself. He concludes that the active principle is neither noradrenaline nor adrenaline, but an unknown orthodiphenol. Östlund (1954) has shown that extracts of insects contain a considerable amount of dopamine also. The present investigation shows that a feeble chromaffine reaction is discernible in the corpus cardiacum of *Iphita*. Histochemical methods for revealing diphenols have, however, yielded only negative results. Chromatograms have shown no definite spots and it may be possible that these are due to the very small quantities of the diphenols that may be present.

Knowles, Carlisle and DuPont-Raåbe (1955) in their studies on the chrome-activating substances in arthropods have shown that in paper electrophoresis of the corpora cardiaca of *Carausius*, elute of a band at -1 was strongly active on the small and large red chromatophores of *Leander*. In electrophoretic migration and in its effects it was comparable to their 'A' substance obtained from extracts of the post-commissural organs and sinus glands of *Leander*. Electrophoresis of brain extracts of *Carausius* showed a substance of low mobility (+0 and -0) which they call 'C' substance, which have no action on chromatophores of *Leander*. The red chromatophores of *Caridina* resemble those of *Leander* in their reactions to extracts of the corpora cardiaca.

Holmes (1950) in discussing chromaffine tests has drawn attention to certain pitfalls in interpretation. He cites Lison's observations that positive chromaffine reaction manifests as a uniform, non-granular cytoplasmic coloration. He points out that prolonged treatment with dichromate and chrome-fixation leads to coloured granular deposits in the cytoplasm. It should be mentioned, however, that yellowish-brown granular substances seen in the cytoplasm of the cells of the corpus cardiacum of *Aspongopus*, where no treatment with dichromate has been done, are probably indicative of a granular chromaffin substance. The glands were here treated with 10 per cent potassium iodate for 48 hours and were then treated with formalin for 24 hours and mounted (Hillarp and Hökfelt method). Such a granular substance was not noticed in *Iphita*.

Ten Cate has observed (Prosser, 1950) that isolated foregut and intestine of *Dytiscus* are excited by acetylcholine and nicotine, while adrenalin has no effect on them. Lewis (1953) has shown that a rapid synthesis of acetylcholine-like substances can take place in extracts of heads of *Calliphora* and *Lucilia* (6.4 and 6.3

$\mu\text{gm./gm.}$ tissue freshweight respectively). But Wigglesworth (1954) has pointed out that acetylcholine has not the stability of the active material from insectan corpora cardiaca and he presumes that it may be related to adrenalin.

5. SUMMARY

1. The corpora cardiaca of *Iphita limbata* Stål. (Pyrrhocoridae: Hemiptera) are paired glands measuring from 182 to 195 μ in diameter.
2. They are connected to the neurosecretory cells of the pars intercerebralis of the brain by the axons of the latter which comprise the cardiac nerve.
3. Each gland is composed of larger peripheral cells situated laterally, which are osmiophil, and smaller cells with heterogeneous cytoplasm. The axons of the cardiac nerve bring neurosecretory colloids and a good quantity of these remains stored in the gland. Phase-contrast and dark-field microscopy, Gomori's chrome-alum-haematoxylin-phloxine and Baker's tests for phospholipids confirm this point.
4. The cells of the corpus cardiacum also elaborate a secretion which appears to be different from the neurosecretory matter.
5. Faint indications of the presence of chromaffin material are given by Lison's tests and indole reaction. Other tests for phenolic substances, including chromatographic analyses, yielded no positive results.
6. The corpus cardiacum contains active substance or substances having a myotropic activity. The extracts from the glands can accelerate the rate of peristalsis of the hindgut of the bug *Aspongopus janus* (Fabr.) (Pentatomidae). Optimum results are obtained at pH 6.0 and a dilution of 1 in 80,000 can induce this acceleration.
7. The extracts of the corpus cardiacum have chromatophoretic effects on the red pigment cells of the Decapod Crustacean *Caridina laevis* Heller and the melanophores of light-adapted and hypophysectomized lizard *Hemidactylus brookii*.
8. The neurosecretory cells seem to have no myotropic or chromatophoretic activity.

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CLASSIFICATION OF THE VEGETATION OF INDIA, PAKISTAN AND BURMA ACCORDING TO EFFECTIVE PRECIPITATION*

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Soon after Koppen's (1900) first attempt to classify the climates of the world on the basis of drainage patterns peculiar to arid regions, many attempts have been made by several investigators in different fields to improve upon his classification. Among these may be mentioned Livingston's (1913, 1916) 'temperature indices', Lang's (1915) ' $P-T$ ratio', De Martonne's (1926) 'index of aridity', Transeau's (1905) 'index of precipitation effectiveness', Meyer's (1926) 'le quotient hygrometrique', Emberger's (1930) 'expression synthetique du climat', Thornthwaite's (1931, 1933) ' $P-E$ indices' which he (1948) later modified into what is at present called the 'potential evapotranspiration'.

In the present investigation an attempt was made to find if the vegetation types of India, Pakistan and Burma as laid down by Champion (1936) could be satisfactorily explained on the basis of Koppen's (1918, 1936) and Thornthwaite's (1931, 1948) climatic classifications. As a result of this it was found that Koppen's as well as Thornthwaite's classifications would not suit the diverse climatic conditions of India, Pakistan and Burma.

Koppen (1900, 1918) published two classifications of the climates of the world, the first in 1900 and the second in 1918. In both these articles he devoted little space to the consideration of the identification of the natural climatic regions. He rather concentrated mainly in developing rules and formulæ for defining De Candolle's (1856) groups in numerical terms. De Candolle (1878) proposed an original classification and proposed also six sub-divisions. Five comprise plants that are physiologically adjusted to various ranges of mean annual temperature and the sixth comprises plants that have made various physiological adaptations to drought. These are the Megistotherms, Megatherms, Mesotherms, Microtherms, Hekistotherms and Xerophytes. De Candolle made use of the symbols A, B, C, D, E to represent his five vegetation groups with B referring to the Xerophytes.

Koppen stated that he had obtained much help and inspiration from Grisebach's (1875) vegetation map and that it had given him an appreciation of the regularity and symmetry of the pattern of climate over the earth. Actually the map is far too general to have been of any direct aid in locating climatic boundaries. Furthermore, there is almost complete lack of accord except in most general terms between Grisebach's map and De Candolle's physiological groups. There is nothing inconsistent in De Candolle's use of the symbols A, B, C, D, E to represent his five vegetation groups since he regarded them as parallel zones but for Koppen to adapt this system to represent the climatic pattern, displayed on Grisebach's map, is quite different. Climatologists have often been puzzled by Koppen's use of the symbol B for the dry climate, not realizing that it traces back through De Candolle to the Greek system now pretty thoroughly discredited.

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Had Koppen used Schimper's (1903) map of vegetation formations as a basis for the identification of climates, his classification would have been very different and very much better. Schimper's vegetation formations are clearly described, easily recognized, and reasonably well mapped. Furthermore, his careful and systematic discussion of the climate corresponding to each formation supplies precisely the material that is needed to work out the general framework of a climatic classification in line with Koppen's ideas. Schimper's work, therefore, provides a basis for a natural classification of climates.

For establishing the boundaries between dry, steppe and humid climates, Koppen did not take into consideration the increase of evaporation with the increase in temperature. Trabert (1896) considers the following formula as the best expression for the rate of evaporation (R):—

$$R = C(I + \alpha t) \sqrt{W} (E - e) \quad \dots \quad (1)$$

In this expression C is a constant depending upon the barometric pressure, W is the wind velocity, E is the maximum vapour pressure for the temperature of the evaporating surface, e is the actual vapour pressure in the air above and $\alpha = 1/273$.

When we substitute the corresponding values for the temperatures 5° , 10° and 15°C ., considering C , W and e as constants for the region under consideration, we find:—

$$R_5 = C(278/273) \sqrt{W} (6.5 - e)$$

$$R_{10} = C(283/273) \sqrt{W} (9.2 - e)$$

$$R_{15} = C(288/273) \sqrt{W} (12.8 - e)$$

This greater increase in the value of R as temperature increases is not expressed in Koppen's formula.

Thornthwaite put forward two classifications of the climate, one in 1931 which he first of all applied to North America and later to the earth, while the second one appeared in 1948. The first classification is based on the computation of P/E ratios in terms of precipitation and temperature of a station. In deriving the relation between P/E and P/T he used evaporation measurements made at twenty-one continental stations which are of one special climatic type (except one coastal station, Crowley, La of which the effect no doubt disappeared in the process of averaging) and hence its applicability elsewhere is rather doubtful. His formula $P/E = 11.5 \left(\frac{P}{T-10} \right)^{10/9}$ when solved for E (the rate of evaporation), gives:—

$$E = 0.087 \frac{(T-10)^{10/9}}{(P)^{1/9}}.$$

In other words, the rate of evaporation varies directly as the approximate temperature and has a small inverse dependence upon precipitation. It is true that the rate of evaporation depends on temperature but it is also controlled by other equally important meteorological factors such as the wind velocity and the saturation deficit, neither of which is allowed for in the formula. Secondly, the presence of precipitation in the above relation is quite unjustifiable, for the rate of evaporation cannot be regarded as a function of precipitation.

As regards Thornthwaite's $T-E$ indices, it may be said that the variations in the heat factor of climate do not generally result in the development of sharply defined boundaries between vegetation formations. As we go from the equator towards either the north or the south, we find that there is a gradual reduction in the variety of flora, with certain species being replaced by others. Thus the boundaries separating tropical, mesothermal, microthermal, taiga and tundra are vague and ill-defined and there is no indication at present that it will be possible to locate them with precision.

The second classification of Thornthwaite's (1948) is based on the computation of potential evapotranspiration by a formula which is purely empirical and according to his own words it does not accord with the newly developed law of growth. Furthermore, the equation completely lacks in mathematical elegance. It is very complicated and, without nomograms and tables as computing aids, would be quite unworkable. The chief obstacle at present to the development of a rational equation is the lack of understanding of why the potential evapotranspiration corresponding to a given temperature is not the same everywhere.

To overcome the above stated defects in the classifications of Koppen as well as those of Thornthwaite, we thought that the P/E ratios based on actual evaporation data should be computed which might enable us to explain the vegetation types of India, Pakistan and Burma according to Champion's classification. It is surprising to note that records of evaporation in various parts of the world are extremely scanty and mostly unsuitable for a comparative study. Both in the length of the records and in the apparatus used for the measurements, there is considerable variation; one may say that, barring a few exceptions, the subject has attracted only amateur attention. Systematic measurements have been started in the United States of America and Egypt; Japan and other countries are slowly following their examples.

In India there are about 3,000 rain-gauge stations, distributed more or less uniformly. The rainfall records of these stations are available generally for more than 60 to 70 years. As compared to this rainfall measurement organization, the attempts at estimating evaporation have been quite meagre owing perhaps to the difficulties of installing suitable instruments and taking observations with them. Some observations spread over a few years are available from the records of the Trivandrum and Madras Observatories. The observations made by Leather (1913) at Pusa, those made at few irrigation works on the Cauvery and at some of the agricultural stations in the Punjab and in Sind and the very detailed observations made at Colaba and discussed by Banerji and Wadia (1932) practically exhaust the scanty list of data available in India. In recent years the Agricultural Meteorological Department have started taking observations of the rate of evaporation using evaporating pans of the U.S. Weather Bureau type but the data are not yet standardized nor are they made available to the public for any investigation. Hence, it became necessary for us to calculate the mean monthly evaporation from other meteorological factors which directly control it.

Experiments carried out in the laboratories and outside have shown that the rate of evaporation increases with (a) the defect of saturation of water vapour, (b) the wind velocity, (c) the temperature of the water surface, and (d) the temperature of the air above the water surface. The effect of atmospheric pressure, judged from simultaneous observations at a few stations at different altitudes, has been found to suppress evaporation with increase of air pressure and vice versa.

There are several formulae put forward by different workers for calculating the rate of evaporation from the meteorological factors but Carl Rohwer's (1931) formula obtained from a long series of elaborate experiments with a view to find separately the influences of each factor such as (1) temperature of the water surface, (2) temperature of the air near the evaporating surface, (3) the effect of saturation, (4) wind velocity near the evaporating surface and (5) altitude of the observation station above mean sea-level has been carefully estimated by a series of experiments. Evaporation figures obtained by his formula are found to be in close agreement with observations made with several types of evaporimeters. Hence it was thought desirable to compute from his formula the mean monthly evaporation needed for our investigation.

The expression given by Rohwer for computing E , the evaporation in inches per 24 hours, is:—

$$E = (1.465 - 0.0186B)(0.44 + 0.118W) (e_s - e_a)$$

where B is the barometric reading in inches of mercury, W , the mean velocity of ground wind in miles per hour, e_s and e_d refer to the mean vapour pressures of the saturated air at the temperatures of the water surface and at dew point respectively, both being measured in inches of mercury. As we have no data of water surface temperatures at our meteorological stations and hence have no means of directly calculating e_s and since we are concerned with the mean daily evaporation, we may assume that the mean daily temperature of the water surface will not differ from that of the air at the same level. On the basis of this assumption Raman and Satakopan (1934) substituted:—

$$\left(\frac{100}{h} - 1\right)e \text{ for } (e_s - e_d)$$

where h is the humidity percentage and e is the vapour pressure. Thus the final expression used for computing the monthly evaporation is:—

$$E = (1.465 - 0.0186B)(0.44 + 0.118W) \left(\frac{100}{h} - 1\right)e.$$

The above formula could not be directly applied to the data recorded by our meteorological stations since the wind instruments of the various stations are usually exposed at the top of a building or a tower, in order to secure as free an exposure as possible. The exposure as well as the height of the instrument above the ground vary considerably from place to place while W in the above expression refers to mean velocity of ground wind in miles per hour.

The variation of wind with height had been studied by Chapman (1932) and from his data it is possible to calculate the ratio of the wind at any height to that at standard level of 4 feet which is the height of the base of the Stevenson screen. This height of 4 feet was chosen since the temperature and humidity data refer to this level and because we have no knowledge at present of the correction for reducing them to any lower level.

To test the applicability of our method, we chose a very large area, namely India, Pakistan and Burma. However, we could only select 104 stations from this vast area since complete and continuous mean monthly records for 15 years (1926–1940) of the seven meteorological elements, namely (1) temperature, (2) precipitation, (3) barometric pressure, (4) wind velocity, (5) percentage humidity, (6) vapour pressure and (7) number of rainy days, together with the characteristic vegetation of other places were not available. Table I gives the statistics regarding the 104 stations from the point of view of availability of water in their vicinity. From this table it is clear that equal importance is given to places in the interior as well as those in the proximity of large bodies of water, which is not considered by Thornthwaite in his earlier classification.

TABLE I

Situatid						
	In the interior	On the West coast	On the East coast	On the banks of the river	On the hills	Total
Number of stations ..	49	12	13	26	4	104

Table II gives the names of the stations that could be included in the present investigation, together with their characteristic vegetation according to Champion, their climatic types according to Koppen's classification of 1918 and 1936, their climatic types according to Thornthwaite's classification of 1931 and 1948, and their P/E indices, which is the sum of the twelve monthly P/E ratios multiplied by 10. The P/E ratios required to get the P/E indices were obtained for each month and for each station using the monthly normals of precipitation and other monthly normal data needed to compute the evaporation from the above modified equation of Carl Rohwer. The monthly normals refer to the period of 15 years already referred to on page 188. The P/E indices in Table II are shown with a subscript outside the bracket. These subscripts refer to the number of months during which there was some measurable precipitation at the station under consideration.

A close examination of Table II shows that both classifications of Koppen's and Thornthwaite's fail to place the same stations having the same characteristic vegetation in the same category of climate. Thus, for example, the first 25 stations in Table II with thorn forest as their characteristic vegetation belong to the semi-arid region of India, Pakistan and Burma. Koppen's classification of 1918 places only 18 of them into the category of 'semi-arid' while the remaining seven fall into three different categories, viz. arid, periodically dry savanna and warm temperate rainy climate with dry winter. His later classification, however, places 22 stations out of these 25 into the semi-arid type whereas the remaining three fall into two different types, viz. desert and periodically dry savanna. Thornthwaite's classifications of 1931 and 1948 place only 17 and 20 of these stations into the semi-arid type while the remaining 8 and 5 stations fall into 4 and 2 different types respectively. Thus, Koppen's classification of 1936 fits better to the semi-arid regions of India, Pakistan and Burma but it does not give satisfactory results when we consider stations in the other groups.

The P/E indices given in Table II of all the stations from Hyderabad (Sind) to Chitaldurg whose climate is akin to that of semi-arid and which have similar vegetation, viz. tropical thorn forest, range between 1 and 5. Places from Madura to Bombay having tropical dry deciduous as their characteristic vegetation have P/E indices which fall in the range from 5 onwards to 15; while stations from Daltonganj to Naya-Dumka having the same characteristic tropical moist deciduous (sal) forest fall in the range of 15 to 20. The tropical semi-evergreen group of stations come in the range of 20 to 50 and places having tropical wet evergreen vegetation have P/E indices above 50. The exceptions to this rule are Port Blair, Kodaikanal and Sagar Island which have tropical wet evergreen, sub-tropical wet and tidal forests respectively as their characteristic vegetation. On the basis of P/E indices these places should have tropical semi-evergreen, moist deciduous (sal) and dry deciduous forests respectively as their natural vegetation but it is not so owing to the peculiar micro-climates of these places. Thus with the exception of the vegetation of these three stations, the vegetation of the remaining 101 stations can be explained on the basis of the calculated P/E indices and grouping them in the way stated below. P/E indices 1-5 thorn forest, 6-15 tropical dry deciduous, 16-20 tropical moist deciduous (sal), 21-50 tropical semi-evergreen, 51 onwards tropical wet evergreen.

These P/E indices have some advantages compared with the aridity factor given by Gorczynski (1942, 1945) and the P/E indices of Thornthwaite. They are:

1. The P/E indices are simple climatic factors and are easier to determine as compared to the aridity index of Gorczynski.

2. The calculation of the P/E indices does not require many years of observations as it is the case with the aridity factor. On the contrary, it can be determined for short periods consecutively, showing interesting fluctuations of climate.

3. Besides precipitation and temperature which are used by Thornthwaite in his computation of the P/E indices, they take into consideration other climatic

TABLE II

Station	Characteristic vegetation according to Champion	Climatic type according to Koppen's classification of		Climatic type according to Thornthwaite's classification of		Computed P/E indices
		1918	1936	1931	1948	
1	2	3	4	5	6	7
Hyderabad (Sind)	Tropical thorn forest	Arid (desert) climate (BW _{hw})	Arid (desert) climate (BW _{hw})	Mesothermal desert climate (EB' wa)	Megathermal, arid climate (EA' da')	(1.187) ₁₂
Gulbarga	"	Periodically dry savanna climate (Aw)	Semi-arid (steppe) climate (BShw)	Tropical grassland (CA' wa)	Megathermal semi-arid (DA' da')	(4.085) ₁₂
Bhuj	"	Semi-arid climate (steppe) (BShw)	Semi-arid (steppe) (BShw)	Tropical semi-arid (steppe) (DA' wa)	Megathermal arid climate (EA' da')	(1.957) ₁₁
Jodhpur	"	"	Arid (desert) climate (BW _{hw})	Mesothermal semi-arid (steppe) (DB' wa)	"	(2.052) ₁₂
Dwarka	"	"	Semi-arid (steppe) (BShw)	Tropical semi-arid (DA' wa)	"	(2.577) ₁₂
Malegaon	"	"	"	Mesothermal semi-arid (DB' wa)	Megathermal semi-arid (DA' da')	(2.598) ₁₂
Bellary	"	"	"	Tropical semi-arid (DA' wa)	"	(2.824) ₁₂
Ahmadnagar	"	"	"	Mesothermal forest (DB' wa)	"	(2.944) ₁₁
Rajkot	"	"	"	Tropical semi-arid (DA' wa)	"	(3.071) ₁₂
Jhansi	"	Warm temperate rainy climate with dry winter (Cwa)	"	Tropical grassland (CA' wa)	Megathermal semi-arid (DA' db ₄)	(4.256) ₁₂
Sholapur	"	Semi-arid (steppe) (BShw)	"	Tropical semi-arid (DA' wa)	Megathermal semi-arid (DA' da')	(3.325) ₁₂

TABLE II—*contd.*

Station	Characteristic vegetation according to Champion	Climatic type according to Köppen's classification of		Climatic type according to Thornthwaite's classification of		Computed P/E indices
		1918	1936	1931	1948	
1	2	3	4	5	6	7
Kurnool	Tropical thorn forest	Semi-arid (steppe) (BShw)	Semi-arid (steppe) (BShw)	Tropical semi-arid (DA' wa)	Megathermal semi-arid (DA' da')	(3.502) ₁₂
Ajmer	"	"	"	Mesothermal semi-arid (DB' wa)	"	(3.500) ₁₂
Agra	"	"	"	"	"	(3.874) ₁₂
Poona	"	Warm temperate rainy climate with dry winter (Cwa)	"	Mesothermal grassland (CB' wa)	Megathermal dry sub-humid (C ₁ B' da')	(4.605) ₁₁
Kotah	"	"	"	Tropical semi-arid (DA' wa)	Megathermal semi-arid (DA' db ₄)	(4.115) ₁₂
Khandwa	"	Semi-arid (steppe) (BShw)	"	Mesothermal grassland (CB' wa)	Megathermal semi-arid (DA' da')	(4.323) ₁₂
Akola	"	Warm temperate rainy climate with dry winter (Cwa)	"	Tropical grassland (CA' wa)	"	(4.355) ₁₂
Veraval	"	Semi-arid (steppe) (BShw)	"	Tropical semi-arid (DA' wa)	"	(4.339) ₁₀
Bhavnagar	"	"	"	"	"	(4.330) ₁₂
Jaipur	"	"	"	Mesothermal semi-arid (DB' wa)	"	(4.187) ₁₂
Deesa	"	"	"	"	"	(4.520) ₁₂
Raichur	"	"	"	Tropical semi-arid (DA' wa)	"	(2.869) ₁₂
Chitaldurg	"	"	"	Mesothermal semi-arid (DB' wa)	"	(4.946) ₁₂
Hanamkonda	"	Periodically dry savanna climate (Aw)	Periodically dry savanna climate (Aw)	Tropical grassland (CA' wa)	"	(4.601) ₁₂
Madura	(Tropical dry deciduous)	"	"	"	"	(5.519) ₁₂

TABLE II—*contd.*

Station	Characteristic vegetation according to Champion	Climatic type according to Koppen's classification of			Climatic type according to Thornthwaite's classification of		Computed P/E indices
		1918	1936	1931	1948		
1	2	3	4	5	6	7	
Neemuch	(Tropical dry deciduous)	Warm temperate rainy climate with dry winter (Cwa)	Warm temperate rainy climate with dry winter (Cwa)	Mesothermal grassland (CB' wa)	Megathermal dry sub-humid (C ₁ A' wa')	(5.565) ₁₂	
Pamban	"	Periodically dry savanna climate (Aw)	Periodically dry savanna climate (Aw)	Tropical grassland (CA' sa)	Megathermal semi-arid (DA' da')	(5.801) ₁₂	
Trichinopoly	"	"	Semi-arid (steppe) (BShw)	Tropical grassland (CA' wa)	"	(6.037) ₁₂	
Amraoti	"	"	Periodically dry savanna climate (Aw)	"	"	(6.139) ₁₂	
Ahmadabad	"	Warm temperate rainy climate with dry winter (Cwa)	Semi-arid (steppe) (BShw)	"	"	(6.239) ₁₁	
Hyderabad (Deccan)	"	"	Warm temperate rainy climate with dry winter (Cwa)	Mesothermal grassland (CB' wa)	Megathermal dry sub-humid (C ₁ A' wa')	(6.406) ₁₂	
Mainpuri	"	"	Semi-arid (steppe) (BShw)	"	Megathermal semi-arid (DA' da')	(6.981) ₁₂	
Indore	"	"	"	"	"	(7.532) ₁₂	
Allahabad	"	"	Warm temperate rainy climate with dry winter (Cwa)	"	Megathermal dry sub-humid (C ₁ A' da')	(7.821) ₁₂	
Mandalay	"	"	"	Tropical grassland (CA' wa)	Megathermal semi-arid (DA' da')	(7.817) ₁₂	

TABLE II—*contd.*

Station	Characteristic vegetation according to Champion	Climatic type according to Koppen's classification of		Climatic type according to Thornthwaite's classification of		Computed P/E indices
		1918	1936	1931	1948	
1	2	3	4	5	6	7
Nizamabad	..	Warm temperate rainy climate with dry winter (Cwa)	Warm temperate rainy climate with dry winter (Cwa)	Tropical grassland (CA' wa)	Megathermal dry sub-humid (C ₁ A' wa')	(8.117) ₁₂
Cawnpur	..	"	"	Mesothermal grassland (CB' wa)	Megathermal semi-arid (DA' da')	(8.697) ₁₂
Salem	..	Periodically dry savanna climate (Aw)	Periodically dry savanna climate (Aw)	Tropical grassland (CA' wa)	"	(8.848) ₁₂
Gaya	..	Warm temperate rainy climate with dry winter (Cwa)	Warm temperate rainy climate with dry winter (Cwa)	Tropical grassland (CA' wa)	Megathermal dry sub-humid (C ₁ A' wa')	(9.251) ₁₁
Bangalore	..	"	"	Mesothermal grassland (CB' wa)	Mesothermal dry sub-humid (C ₁ B' wa')	(9.177) ₁₂
Chanda	..	"	"	Tropical forest (BA' wa)	Megathermal moist sub-humid (C ₂ A' wa')	(9.302) ₁₂
Ambala	..	Warm temperate rainy climate without dry season (Cfa)	Warm temperate rainy climate without dry season (Cfa)	Mesothermal grassland (CB' wb)	Megathermal semi-arid (DA' db')	(9.515) ₁₂
Nagpur	..	Warm temperate rainy climate with dry winter (Cwa)	Warm temperate rainy climate with dry winter (Cwa)	Tropical grassland (CA' wa)	Megathermal dry sub-humid (C ₁ A' wa')	(9.926) ₁₂
Surat	..	"	"	"	Megathermal dry sub-humid (C ₁ A' wa')	(10.116) ₁₁

TABLE II—contd.

Station	Characteristic vegetation according to Champion	Climatic type according to Koppen's classification of		Climatic type according to Thornthwaite's classification of		Computed P/E indices
		1918	1936	1931	1948	
1	2	3	4	5	6	7
Lucknow	(Tropical dry deciduous)	Warm temperate rainy climate with dry winter (Cwa)	Warm temperate rainy climate with dry winter (Cwa)	Mesothermal grassland (CB' wa)	Megathermal dry sub-humid (C ₁ A' da')	(10-132) ₁₂
Nellore	"	Periodically dry savanna climate (Aw)	Periodically dry savanna climate (Aw)	Tropical grassland (CA' sa)	Megathermal semi-arid (DA' da')	(10-488) ₁₂
Hazari bag	"	Warm temperate rainy climate with dry winter (Cwa)	Warm temperate rainy climate with dry winter (Cwa)	Mesothermal forest (BB' wa)	Megathermal humid (B ₁ A' w ₂ a')	(11-247) ₁₂
Benares	"	"	"	Tropical forest (BA' wa)	Megathermal dry sub-humid (C ₁ A' da')	(11-247) ₁₂
Monywa	"	"	"	Tropical grassland (CA' wa)	Megathermal semi-arid (DA' da')	(11-428) ₁₀
Sutna	"	"	"	Mesothermal grassland (CB' wa)	Megathermal dry sub-humid (C ₁ A' wb' ₄)	(11-845) ₁₂
Cuddalore	"	Periodically dry savanna climate (Aw)	Periodically dry savanna climate (Aw)	Tropical forest (BA' sa)	Megathermal dry sub-humid (C ₁ A' s ₂ a')	(12-672) ₁₂
Bhagalpur	"	"	"	Tropical forest (BA' wa)	Megathermal dry sub-humid (C ₁ A' wa')	(12-956) ₁₁
Patna	"	"	"	"	Megathermal moist sub-humid (C ₂ A' wa')	(12-973) ₁₂
Raipur	"	"	"	Mesothermal forest (BB' wa)	Megathermal moist sub-humid (C ₂ A' w ₂ b' ₄)	(13-483) ₁₂

TABLE II—contd.

Station	Characteristic vegetation according to Champion	Climatic type according to Koppen's classification of		Climatic type according to Thornthwaite's classification of		Computed P/E indices
		1918	1936	1931	1948	
1	2	3	4	5	6	7
Seoni	(Tropical dry deciduous)	Periodically dry savanna climate (Aw)	Periodically dry savanna climate (Aw)	Mesothermal forest (BB' wa)	Megathermal moist sub-humid (C ₂ A' w ₂ b' a)	(13.741) ₁₂
Madras	"	"	"	Tropical forest (BA' sa)	Megathermal dry sub-humid (C ₁ A' sa')	(13.703) ₁₂
Bahraich	"	Warm temperate rainy climate with dry winter (Cwa)	Warm temperate rainy climate with dry winter (Cwa)	Mesothermal grassland (CB' wa)	Megathermal dry sub-humid (C ₁ A' wa')	(13.922) ₁₂
Hosangabad	"	"	"	"	Megathermal moist sub-humid (C ₂ A' s ₂ b' a)	(14.099) ₁₂
Yamethin	"	"	"	Tropical grassland (CA' wa)	Megathermal semi-arid (DA' da')	(14.676) ₁₂
Bombay	"	Periodically dry savanna climate (Aw)	Periodically dry savanna climate (Aw)	Tropical forest (BA' wa)	Megathermal humid (B ₁ A' w ₂ a')	(14.760) ₁₁
Daltonganj	Tropical moist deciduous (sal)	Warm temperate rainy climate with dry winter (Cwa)	Warm temperate rainy climate with dry winter (Cwa)	Mesothermal grassland (CB' wa)	Megathermal dry sub-humid (C ₁ A' da')	(15.167) ₁₂
Sambalpur	"	"	"	Tropical forest (BA' wa)	Megathermal moist sub-humid (C ₂ A' s ₂ a')	(15.557) ₁₂
Ranchi	"	"	"	Mesothermal forest (BB' wa)	Megathermal humid (B ₁ A' sa')	(15.587) ₁₂
Darbhang	"	"	"	"	Megathermal moist sub-humid (C ₂ A' sa')	(16.199) ₁₂

TABLE II—*contd.*

Station	Characteristic vegetation according to Champion	Climatic type according to Koppen's classification of			Climatic type according to Thornthwaite's classification of		Computed P/E indices
		1918	1936	1931	1948		
1	2	3	4	5	6	7	
Bareilly	Tropical moist deciduous (sal)	Warm temperate rainy climate with dry winter (Cwa)	Warm temperate rainy climate with dry winter (Cwa)	Mesothermal grassland (CB' wa)	Megathermal dry sub-humid (C_1A' wb' $_4$)	(16.847) ₁₂	
Jubbulpur	"	"	"	Mesothermal forest (BB' wa)	Megathermal moist sub-humid (C_2A' s $_2$ b' $_4$)	(18.084) ₁₂	
Gorakhpur	"	"	"	Mesothermal grassland (CB' wa)	Megathermal moist sub-humid (C_2A' sa')	(18.339) ₁₂	
Balasore	"	"	"	Tropical forest (BA' wa)	"	(18.419) ₁₂	
Naya-Dumka	"	"	"	Mesothermal forest (BB' wa)	"	(19.612) ₁₂	
Ratnagiri	Tropical semi-evergreen	Periodically dry savanna climate (Aw)	Periodically dry savanna climate (Aw)	Tropical rain forest (AA' wa)	Megathermal humid (B_3A' w $_2$ a')	(20.540) ₁₂	
Trivandrum	"	"	"	Tropical forest (BA' wa)	Megathermal humid (B_1A' sa')	(20.631) ₁₂	
Cuttack	"	Warm temperate rainy climate with dry winter (Cwa)	Warm temperate rainy climate with dry winter (Cwa)	"	Megathermal moist sub-humid (C_2A' sa')	(21.279) ₁₂	
Belgaum	"	Periodically dry savanna climate (Aw)	Periodically dry savanna climate (Aw)	Mesothermal forest (BB' wa)	Mesothermal humid (B_2B' s $_2$ a')	(21.389) ₁₁	
Berhampore	"	Warm temperate rainy climate with dry winter (Cwa)	Warm temperate rainy climate with dry winter (Cwa)	"	Megathermal humid (B_1A' sa')	(22.146) ₁₂	

TABLE II—*contd.*

Station	Characteristic vegetation according to Champion	Climatic type according to Koppen's classification of			Climatic type according to Thornthwaite's classification of		Computed P/E indices
		1918	1936	1931	1948		
1	2	3	4	5	6	7	
Narayanganj	Tropical semi-evergreen	Warm temperate rainy climate with dry winter (Cwa)	Warm temperate rainy climate with dry winter (Cwa)	Tropical forest (BA' wa)	Megathermal humid (B ₂ A' ra')	(26.778) ₁₂	
Cochin	"	Periodically dry savanna climate (Aw)	Periodically dry savanna climate (Aw)	Tropical rain forest (AA' wa)	Megathermal humid (B ₄ A' ra')	(29.884) ₁₂	
Purnea	"	Warm temperate rainy climate with dry winter (Cwa)	Warm temperate rainy climate with dry winter (Cwa)	Mesothermal forest (BB' wa)	Megathermal humid (B ₁ A' ra')	(30.546) ₁₂	
Marmagao	"	Periodically dry savanna climate (Aw)	Periodically dry savanna climate (Aw)	Tropical rain forest (AA' wa)	Megathermal humid (B ₃ A' s ₂ a')	(31.691) ₁₀	
Bogra	"	Warm temperate rainy climate with dry winter (Cwa)	Warm temperate rainy climate with dry winter (Cwa)	Mesothermal forest (BB' wa)	Megathermal humid (B ₂ A' ra')	(31.973) ₁₂	
Gauhati	"	"	"	"	Megathermal humid (B ₁ A' ra')	(34.354) ₁₂	
Shillong	"	Warm temperate rainy climate with dry winter (Cwb)	Warm temperate rainy climate with dry winter (Cwb)	Mesothermal rain forest (AB' wa)	Microthermal per humid (AB' s ₂ ra')	(37.368) ₁₂	
Dhubri	"	Warm temperate rainy climate with dry winter (Cwa)	Warm temperate rainy climate with dry winter (Cwa)	"	Megathermal per humid (AA' ra')	(41.722) ₁₂	
Karwar	"	Monsoon type of tropical climate (Am)	Monsoon type of tropical climate (Am)	Tropical rain forest (AA' wa)	Megathermal per humid (AA' s ₂ a')	(45.292) ₁₀	

TABLE II—*contd.*

Station	Characteristic vegetation according to Champion	Climatic type according to Köppen's classification of		Climatic type according to Thornthwaite's classification of		Computed P/E indices
		1918	1936	1931	1948	
1	2	3	4	5	6	7
Tezpur	(Tropical semi-evergreen)	Warm temperate rainy climate with dry winter (Cwa)	Warm temperate rainy climate with dry winter (Cwa)	Mesothermal forest (BB' wa)	Mesothermal humid (B ₃ B' ₄ ra')	(45.287) ₁₂
Mangalore	"	Monsoon type of tropical climate (Ami)	Monsoon type of tropical climate (Ami)	Tropical rain forest (AA' wa)	Megathermal per humid (AA' _{2a} ')	(48.630) ₁₂
Toungoo	Tropical wet evergreen	Warm temperate rainy climate with dry winter (Cwa)	Warm temperate rainy climate with dry winter (Cwa)	"	Megathermal humid (B ₃ A' ra')	(52.537) ₁₂
Rangoon	"	Monsoon type of tropical climate (Ami)	Monsoon type of tropical climate (Ami)	"	Megathermal per humid (AA' ra')	(53.863) ₁₂
Chittagong	"	Warm temperate rainy climate with dry winter (Cwa)	Warm temperate rainy climate with dry winter (Cwa)	Mesothermal rain forest (AB' wa)	"	(55.358) ₁₂
Myitkina	"	"	"	Mesothermal forest (BB' wa)	Mesothermal humid (B ₄ B' ₄ ra')	(60.665) ₁₂
Cox's Bazar	"	"	"	Tropical rain forest (AA' wa)	Megathermal per humid (AA' ra')	(60.237) ₁₂
Sibsagar	"	"	"	Mesothermal rain forest (AB' wa)	Mesothermal per humid (AB' ₄ ra')	(61.840) ₁₂
Silchar	"	"	"	"	Megathermal per humid (AA' ra')	(71.407) ₁₂

TABLE II—*concl.*

Station	Characteristic vegetation according to Champion	Climatic type according to Koppen's classification of		Climatic type according to Thornthwaite's classification of		Computed P/E indices
		1918	1936	1931	1948	
1	2	3	4	5	6	7
Bassein	Tropical wet evergreen	Monsoon type of tropical climate (Ami)	Monsoon type of tropical climate (Ami)	Mesothermal rain forest (AB' wa)	Mesothermal per humid (AB ₄ ' ra')	(70-151) ₁₂
Bhamo	"	Warm temperate rainy climate with dry winter (Cwa)	Warm temperate rainy climate with dry winter (Cwa)	Mesothermal forest (BB' wa)	Mesothermal humid (B ₄ B ₄ ' ra')	(91-180) ₁₂
Mergui	"	Monsoon type of tropical climate (Ami)	Monsoon type of tropical climate (Ami)	Tropical rain forest (AA' wa)	Megathermal per humid (AA' ra')	(130-218) ₁₂
Tavoy	"	"	"	"	Megathermal per humid (AA' sa')	(188-551) ₁₂
Dibrugar	"	Warm temperate rainy climate with dry winter (Cwa)	Warm temperate rainy climate with dry winter (Cwa)	Mesothermal rain forest (AB' wa)	Mesothermal per humid (AB ₄ ' ra')	(145-571) ₁₂
Akyab	"	"	"	Tropical rain forest (AA' wa)	Megathermal per humid (AA' ra')	(190-265) ₁₂
Mercara	"	Warm temperate rainy climate with dry winter (Cwb)	Warm temperate rainy climate with dry winter (Cwb)	Mesothermal rain forest (AB' wa)	Microthermal per humid (AB ₂ ' ra')	(241-743) ₁₂
Cherrapunji	"	"	"	"	"	(447-342) ₁₂
Port Blair	"	Monsoon type of tropical climate (Ami)	Monsoon type of tropical climate (Ami)	Tropical rain forest (AA' ra)	Megathermal humid (B ₃ A' ra')	(25-582) ₁₂
Kodaikanal	Sub-tropical wet forest	Warm temperate rainy climate without dry season (Cfb)	Warm temperate rainy climate without dry season (Cfb)	Mesothermal forest (BB' ra)	Microthermal per humid (AB ₁ ' ra')	(17-563) ₁₂
Sagar Island	Tidal	Warm temperate rainy climate with dry winter (Cwa)	Warm temperate rainy climate with dry winter (Cwa)	Tropical forest (BA' wa)	Megathermal moist sub-humid (C ₂ A' wa')	(12-841) ₁₂

factors such as humidity, barometric pressure, wind velocity, etc. The objection that there are not many evaporation data available is not serious, since the evaporation capacity of the air may be easily calculated by a formula using temperature, humidity and wind velocity. Another objection may be raised that the evaporation data are not comparable in the same degree as the rainfall values. Several different methods of evaporation measurements are used indeed, but if we exclude observations executed by means of floating pans for hydrologic purposes and confine ourselves to pans set over the ground, then the difference will not be too great. One should also hope that sooner or later the methods of determining evaporation rates will be standardized to a certain degree.

In our present attempt of explaining the natural vegetation of India, Pakistan and Burma on the basis of P/E ratios, we were required to calculate the evaporation figures of each station for each month of the year. Thus the total number of evaporation figures as well as the P/E ratios amounted to 1,248 (104×12) groups. Hence, it was thought that such a large body of data should first of all be used to test as to whether the relation deduced by Thornthwaite:—

$$P/E = 11.5 \left(\frac{P}{T-10} \right)^{10/9}$$

using only 1,100 groups of P/E ratios, is true in the case of Indian data or not. The procedure adopted to test the validity of the above formula is the same as suggested by Thornthwaite. The calculated P/E ratios were arranged in such a manner that all having similar P/E ratios were put together, the dates and the locations of the P/E ratios being ignored while grouping. In this way 63 groups of P/E ratios whose values varied from 0.01 to 5.4 were obtained. The data were then represented on a graph, where the monthly normal temperature expressed in degree Fahrenheit was plotted along the ordinate and the monthly normal precipitation along the abscissa. Thus 63 separate graphs, one for each group of P/E ratios, were obtained. Instead of drawing a straight line through each of these plots by visual judgement as was done by Thornthwaite, a straight line equation of the form $T = A + BP$ was computed by the method of least squares using the temperature and precipitation data in each plot. In this way 60* straight line equations were computed for the various P/E ratios.

This method of computing a straight line equation is not subjected to criticism as is the case with the method adopted by Thornthwaite. Whereas Thornthwaite's method is a visual one, i.e. to draw a straight line passing through the maximum points in each plot and hence an approximate one, for the nature of the straight line will depend much upon the investigator, the method here is a statistical one and hence the nature of the straight line will not vary with the investigator.

The 60 different equations obtained by the method stated above showed that none of the straight lines which they represent would cut the ordinate at 10°F . as was found to be the case by Thornthwaite. The values of the constants A in the equation which represents the point of intersection of the straight lines with the Y axis and which can be obtained by putting $P = 0$ in each one of the 60 equations were found to vary from a minimum of 19.12°F . in the case of the plot having a P/E ratio 5.1 to a maximum of 95.55°F . in the case of the plot of P/E ratio 5.2. The mean value of these 60 different intercepts was found to be 68.09 . Thus it was found that the result obtained by Thornthwaite, viz. that in the case of each plot a straight line cutting the ordinate at 10°F . fitted the data satisfactorily, was found to be no longer valid with the present data.

* There were in all 63 plots having P/E ratios ranging from 0.01 to 5.4. Therefore there should be 63 straight line equations but as there was only one observation in each one of the plots having P/E ratios 4.7 and 4.8 and no observation in the plot with P/E ratios equal to 5.3, no equation could be fitted for these plots. Therefore, we got only 60 equations for 63 plots.

Lastly, from these several equations and proceeding on the lines suggested by Thornthwaite, an attempt was made to formulate a single equation which expressed the relation of P/E ratios to the easily available meteorological elements, precipitation and temperature alone. As a result of this investigation it was found that there cannot be a simple relation between P/E and P/T , namely

$$P/E = (11.5) \left(\frac{P}{T-10} \right)^{10/9}$$

deduced by Thornthwaite on the visual judgement that the temperature against precipitation curves would be straight lines cutting the ordinates at 10°F .

The computation of the P/E ratios, which explains satisfactorily the vegetation types of India, Pakistan and Burma, from other meteorological factors is very laborious and hence an attempt was made to obtain these P/E ratios in terms of P/T on the lines suggested by Thornthwaite but the attempt did not prove to be successful. Therefore, in our further attempt we proceeded to find out as to whether there is any other easily available meteorological factor which could be correlated with the P/E ratios.

Nearly a century ago De Candolle (1856) had advocated that the number of rainy days are the best expressions for denoting the humidity or aridity of a country. He said, 'the number of days of rain seemed to me to be the best expression of the conditions of humidity or dryness of a country as relating to the vegetation. I prefer this figure to that of the quantity of rain, and it is easier to obtain, I would not know how to recommend it too highly to the meteorological societies, and amateur scientists scattered in every country. Nothing is easier than marking on an almanac (calendar) each twenty-four hour day in which some rain or some snow has fallen. After ten or fifteen years, one can draw upon the data very advantageously to learn the mean number of natural rainfalls per month, or better yet, per decade, the mean duration and extreme of droughts, the mean duration and extreme of precipitation in certain seasons. No instrument is needed for this; good will and exactitude suffices'.

Koppen (1900) while discussing the moisture factor with reference to dry climate said, 'precipitation alone is of little help in determining the moisture content of the soil and especially in distinguishing a moist or dry climate. Here the time and character of the precipitation and the amount of evaporation are decisive. Equal amounts of rainfall, for example, produce primeval forest in Siberia and pronounced desert plants in Africa. This is one reason why I prefer tabulation of the number of rainy days. Actually the boundary between forest and steppe is in general defined climatologically more readily by means of the number of rainy days than by the rainfall amount'.

It is well known that the same amount of rainfall produces a great difference in the vegetation as the rain falls uniformly throughout a long period or falls for only a very short time in the form of heavy storms. The number of rainy days is, therefore, of greater importance than the amount of rain. In the former case the rain is capable of being much more beneficial to the vegetation; in the later case the parched soil is not in a condition to absorb all the water, most of which flooding and denuding the soil, flows over its surface or percolates to its depths. Under the former conditions we find growth-forms and plant communities quite different from those under the later. Hence the number of rainy days appeared to us to be the best and easily available meteorological element which could be used to explain the vegetation of any locality. Therefore, with a view to find out as to whether a relationship exists between the P/E indices and the number of rainy days of a station, the mean annual (mean of fifteen years) number of rainy days (D) were plotted along the ordinate and the P/E indices along the abscissa. A study of the graph showed that a parabola

passing through the origin could be fitted to the data. Hence using the P/E indices and the mean annual number of rainy days of 98* stations a formula of the type

$$P/E = aD + bD^2$$

was computed by the method of least square. The final equation for the data was

$$P/E = (-0.065251)D + (0.004691)D^2$$

which enables one to get the P/E indices and hence the vegetation type of a station from the mere knowledge of its annual number of rainy days.

In conclusion the authors express their acknowledgement to Professor C. Troll of the University of Bonn, W. Germany, for his suggestions.

ABSTRACT

Comparison of the vegetation types of India and vicinity as laid down by Champion with those obtained from theoretical consideration using Koppen's and Thornthwaite's formulae revealed that both the formulae cannot satisfactorily explain the varied natural vegetation types of the Indian subcontinent. A satisfactory explanation of the natural vegetation types could, however, be made on the basis of P/E (mean monthly precipitation/mean monthly evaporation from the free water surface) ratios. The mean monthly evaporation from the free water surface for 104 stations used in the present investigation was calculated from the mean monthly barometric pressure, wind velocity, relative humidity and vapour pressure using Carl Rohwer's formula as modified by Raman and Satakopan. The P/E index which is the sum of the twelve monthly P/E ratios of a station, when grouped in the following way:—indices 1-5—thorn forest, 5-15—tropical dry deciduous, 15-20—tropical moist deciduous, 20-50—tropical semi-evergreen, 50 onwards—tropical wet evergreen, were able to explain the real distribution of the vegetation types. It was further shown that the P/E index of a station, which is very laborious to compute in the usual way, could easily be obtained from the mean annual number of rainy days of that station.

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* Out of 104 stations the data of 98 stations alone was used to compute the equation of the parabola since the co-ordinates of the points of the following six stations showed considerable deviation from the parabola. These stations are:—(1) Sagar Island, (2) Port Blair, (3) Cherrapunji, (4) Mercara, (5) Tavoy and (6) Akyab.

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STUDIES ON CELLULOLYTIC ACTIVITY OF FUSARIA WITH REFERENCE TO BACTERIAL AND OTHER CELLULOSE SUBSTRATES

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INTRODUCTION

In recent years numerous investigations have been carried out on the cellulolytic activity of *Fusarium* species with reference to the part played by these fungi in the decomposition of cotton fabrics under various exposure conditions (White *et al.*, 1948; Marsh and Bollenbacher, 1949; Marsh *et al.*, 1949). Other reports list a few species which were found capable of decomposing cellulose (Thaysen and Bunker, 1937; Siu, 1951). White *et al.* (1948) found twenty-seven isolates of *Fusaria*, out of the thirty-four investigated, to be active in pure-culture cotton fabric tests for cellulolytic activity. These tests were carried out on strips of cotton fabrics and were aimed mainly at indexing the economic importance of the fungi in causing deterioration of the fabrics under field conditions from the point of industrial and military considerations. Evidence has been presented by Tracey (1953) to show that activity of cellulolytic enzyme preparation obtained from fungi is dependent upon the nature of the cellulose substrata. It would seem important, therefore, to test fungal species on different cellulosic substrates in evaluating the cellulolytic activity of the species. So far, no attempt seems to have been made to estimate the activity of fungi on bacterial cellulose membranes biosynthesized by *Acetobacter xylinum*. These membranes are known to be the purest form of cellulose (Gortner, 1949) and have been used in the isolation of cellulose-splitting bacteria from soil (Aschner, 1937). The present investigation was undertaken as a comparative study on the cellulolytic activity of twenty-three species of *Fusarium* on bacterial as well as other cellulose substrates.

MATERIALS AND METHODS

All the species of *Fusaria* employed in this investigation were obtained from the Centraalbureau voor Schimmelcultures, Baarn, Holland, and monoonidial cultures of these species were tested for cellulolytic activity. The inoculum was prepared as follows: Strips of filter paper were placed in half-strength Richards solution (sugar omitted) in a test-tube and autoclaved. In each case, a spore suspension of any one fungus was placed on the protruding but moist portion of the strip and allowed to grow at 25–29°C. for 10 days. After incubation, a portion of the growing colony on the filter paper was used for inoculation in determining cellulolytic activity.

Cellulolytic activity of the species on filter paper: A central core was cut out from discs of Whatman No. 1 filter paper and one such disc (1100 mg.) was folded up in the form of a fluted cone and introduced into 250 ml. conical flasks containing 50 ml. medium in such a way that approximately one-third of the filter paper remained immersed in the solution. After autoclaving, the flasks were inoculated by placing the inoculum on the unsubmerged portion of the paper. The weight of the residual filter paper, left over after fungal decomposition, was determined after 15 and 30 days' incubation at 25–29°C.

Cellulolytic activity of the enzyme preparation: Suspension of regenerated filter paper (Scales, 1915) was taken in Sorensen's phosphate buffer at pH 5.0, 5.5, 6.0, 6.5, 7.0, and 7.5, and in each case, 5 ml. of the culture filtrate, obtained after 3 weeks' growth of different *Fusaria* on filter paper, was added to 5 ml. aliquots of the suspension in test-tubes maintained at 37°C. Turbidometric determinations of the density of the cellulose suspension were made after 0, 4, 16, 22, 28 and 40 hours in Spekker Absorptiometer using H.2 neutral filter. The increase in percentage light transmission plotted against time interval indicated enzymic activity of the filtrate.

Cellulolytic activity of Fusaria on bacterial cellulose: *Acetobacter xylinum* was grown on yeast-extract-sucrose medium (Aschner, 1937) and after 5 days' growth at 27°C., the bacterial membranes were washed thoroughly in tap water, immersed in 20% sodium hydroxide for 2 days to dissolve out the bacterial protein, washed again in running tap water, rinsed in dilute HCl to remove the last traces of the alkali and finally washed again in tap water and distilled water. These membranes were soaked in half-strength Richards mineral solution in flasks, autoclaved and after 2-3 days one such membrane was transferred to a 10-cm. Petri dish and the surface moisture on the membrane removed with a filter paper. The dishes were autoclaved and the membranes inoculated in the centre in a manner similar to that of agar plates. The radial spread of the *Fusaria* on the cellulose membrane (Plate XX, Fig. 9) resulted in the liquefaction of the cellulose and indicated cellulolytic activity of the species. To determine the area of liquefaction, the surface of the membrane covered with the fungal colony was gently rubbed with a glass spatula when the liquefied area separated from the rest of the membrane into a jelly-like mass (Plate XX, Figs. 11 and 12). It was further observed that the area of liquefaction was slightly less than that covered by the fungal growth. Approximately, 2 mm. of the peripheral region of the fungal colony was not liquefied. This was probably due to the fact that enzymic activity in this area had not progressed sufficiently. In the present work, all results are expressed as the diameter of the fungal colony on the cellulose membrane.

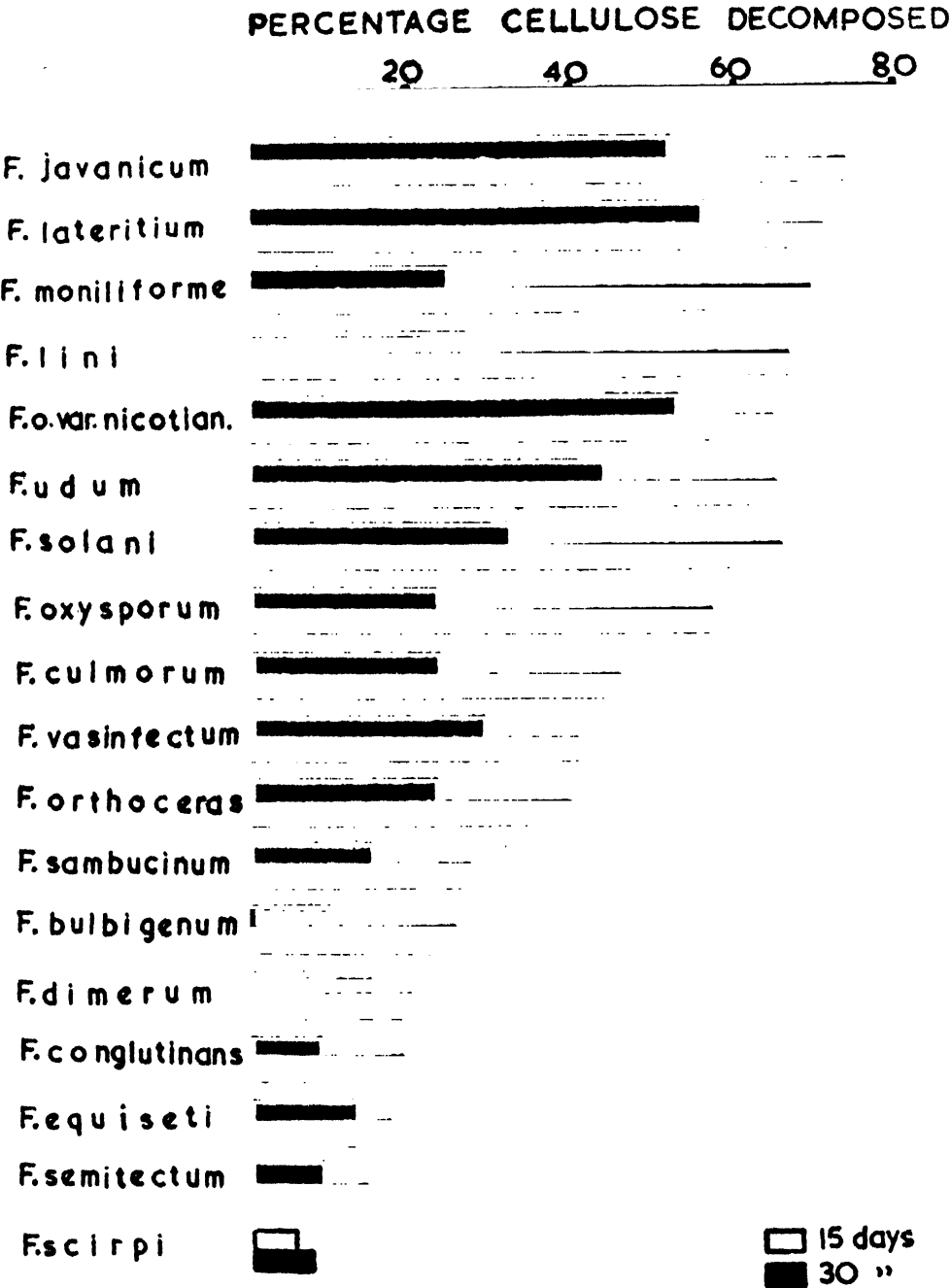
RESULTS

Text-fig. 1 shows the results of the cellulolytic activity of the *Fusaria* on filter paper. No correction has been made here for the weight of the fungal mat and it is deemed that these results would provide sufficient index for the purpose of comparing the cellulolytic potentiality of the different species.

Degradation of cellulose by the *Fusaria* differed strikingly with the species (Plate XX, Figs. 1-8). Whilst most of the *Fusaria* digested filter paper to varying extent, five species, *F. buharicum*, *F. caucasicum*, *F. chlamydosporum*, *F. sporotrichioides* and *F. poae*, did not decompose filter paper. *F. javanicum*, *F. lateritium*, *F. lini*, *F. moniliforme*, *F. solani*, *F. oxysporum* var. *nicotianae*, *F. udum* and *F. oxysporum*, in the order mentioned, were strongly cellulolytic, whereas *F. bulbigenum* var. *lycopersici*, *F. conglutinans*, *F. dimerum*, *F. equiseti*, *F. sambucinum*, *F. scirpi* and *F. semitectum* were poor decomposers of filter paper (Text-fig. 1). *F. vasinfectum*, the cotton wilt pathogen, *F. orthoceras* and *F. culmorum* utilized the cellulose to a moderate extent. Test for glucose in the 15-day-old cultures undergoing degradation of filter paper were negative, but traces of glucose were detected in the metabolic filtrate obtained 30 days after cellulose degradation had progressed.

In correlating cellulolytic activity of the *Fusarium* with its species, the strength of enzyme (cellulase) activity of the filtrate of *Fusaria* growing on filter paper was determined as an index of the cellulolytic activity of the species on filter paper cellulose; results are presented in Text-fig. 2 and Table I.

Excepting *F. chlamydosporum*, cellulase activity was observed in the filtrates of the other species tested. The data obtained here showed that enzymic strength of the metabolic filtrate was directly related to cellulolytic activity of the species on



Text-fig. 1. Cellulolytic activity of Fusaria on filter paper.

filter paper. Filtrates of *F. moniliforme*, *F. javanicum* and *F. lateritium*, which caused maximum breakdown of filter paper (Text-fig. 1), had highest cellulase activity (Text-fig. 2); conversely, low enzyme strength was observed in the filtrates of *F. scirpi* and *F. dimerum*, which were found to be poor decomposers of cellulose. The strength of the enzyme activity of the filtrate varied with the pH of the medium in which it was tested (Text-fig. 2). For the filtrate of most species tested,

TABLE I

Showing enzyme activity of *Fusarium* culture filtrates on re-precipitated cellulose by turbidometric measurements

<i>Fusarium</i> species	pH of medium	Light transmission after					
		0 hr.	4 hr.	16 hr.	22 hr.	28 hr.	40 hr.
<i>F. lateritium</i>	5.0	0.270	0.280	0.325	0.345	0.370	0.405
	5.5	0.268	0.285	0.335	0.360	0.390	0.425
	6.0	0.268	0.280	0.320	0.345	0.378	0.410
	6.5	0.268	0.290	0.315	0.335	0.362	0.388
	7.0	0.265	0.268	0.290	0.308	0.335	0.365
	7.5	0.270	0.270	0.295	0.318	0.341	0.360
<i>F. badhigenum</i> var. <i>lycopersici</i>	5.0	0.270	0.285	0.305	0.335	0.345	0.355
	5.5	0.270	0.290	0.315	0.350	0.365	0.375
	6.0	0.275	0.290	0.300	0.335	0.345	0.360
	6.5	0.260	0.290	0.305	0.320	0.330	0.345
	7.0	0.255	0.275	0.285	0.300	0.312	0.325
	7.5	0.265	0.275	0.285	0.310	0.320	0.335
<i>F. lini</i>	5.0	0.280	0.290	0.305	0.350	0.365	0.395
	5.5	0.285	0.295	0.315	0.368	0.388	0.415
	6.0	0.280	0.290	0.300	0.355	0.375	0.395
	6.5	0.275	0.301	0.310	0.335	0.350	0.365
	7.0	0.270	0.310	0.290	0.320	0.345	0.375
	7.5	0.285	0.285	0.310	0.315	0.330	0.350
<i>F. solani</i>	5.0	0.250	0.315	0.280	0.300	0.310	0.335
	5.5	0.250	0.270	0.290	0.312	0.328	0.335
	6.0	0.260	0.280	0.305	0.330	0.348	0.362
	6.5	0.250	0.275	0.302	0.335	0.365	0.378
	7.0	0.260	0.280	0.305	0.325	0.362	0.370
	7.5	0.255	0.275	0.300	0.325	0.345	0.358
<i>F. vasinfectum</i>	5.0	0.140	0.175	0.205	0.245	0.255	0.275
	5.5	0.145	0.178	0.215	0.258	0.270	0.295
	6.0	0.140	0.165	0.200	0.240	0.255	0.275
	6.5	0.140	0.165	0.205	0.225	0.240	0.255
	7.0	0.145	0.160	0.180	0.220	0.240	0.255
	7.5	0.140	0.140	0.180	0.210	0.215	0.230
<i>F. oxysporum</i>	5.0	0.255	0.280	0.290	0.325	0.350	0.370
	5.5	0.250	0.280	0.295	0.335	0.365	0.385
	6.0	0.250	0.272	0.292	0.325	0.350	0.370
	6.5	0.250	0.270	0.290	0.320	0.335	0.355
	7.0	0.250	0.272	0.290	0.320	0.340	0.355
	7.5	0.250	0.255	0.290	0.320	0.335	0.350
<i>F. equiseti</i>	5.0	0.255	0.290	0.315	0.330	0.355	0.370
	5.5	0.260	0.295	0.318	0.335	0.365	0.385
	6.0	0.255	0.285	0.290	0.320	0.355	0.368
	6.5	0.260	0.280	0.280	0.320	0.345	0.365
	7.0	0.265	0.280	0.290	0.320	0.355	0.368
	7.5	0.270	0.275	0.290	0.320	0.335	0.365
<i>F. acirpi</i>	5.0	0.260	0.260	0.275	0.300	0.310	0.320
	5.5	0.260	0.265	0.278	0.305	0.310	0.320
	6.0	0.260	0.262	0.282	0.310	0.320	0.330
	6.5	0.260	0.270	0.280	0.320	0.328	0.340
	7.0	0.265	0.268	0.268	0.295	0.310	0.335
	7.5	0.270	0.270	0.290	0.310	0.325	0.340

TABLE I—*contd.*

<i>Fusarium</i> species	pH of medium	Light transmission after					
		0 hr.	4 hr.	16 hr.	22 hr.	28 hr.	40 hr.
<i>F. dimerum</i>	5.0	0.260	0.265	0.285	0.310	0.320	0.335
	5.5	0.260	0.265	0.285	0.320	0.325	0.335
	6.0	0.260	0.275	0.290	0.330	0.335	0.345
	6.5	0.255	0.280	0.295	0.320	0.340	0.355
	7.0	0.265	0.275	0.300	0.325	0.340	0.355
	7.5	0.265	0.265	0.295	0.325	0.340	0.355
<i>F. javanicum</i>	5.0	0.150	0.170	0.220	0.235	0.245	0.265
	5.5	0.150	0.170	0.220	0.245	0.255	0.275
	6.0	0.150	0.175	0.230	0.265	0.375	0.290
	6.5	0.155	0.165	0.230	0.280	0.303	0.330
	7.0	0.160	0.185	0.232	0.265	0.290	0.320
	7.5	0.155	0.178	0.235	0.270	0.285	0.305
<i>F. moniliforme</i>	5.0	0.270	0.295	0.330	0.360	0.375	0.410
	5.5	0.270	0.295	0.335	0.375	0.390	0.435
	6.0	0.265	0.290	0.325	0.365	0.385	0.415
	6.5	0.270	0.290	0.328	0.365	0.380	0.398
	7.0	0.270	0.285	0.315	0.345	0.365	0.390
	7.5	0.270	0.275	0.315	0.340	0.355	0.375

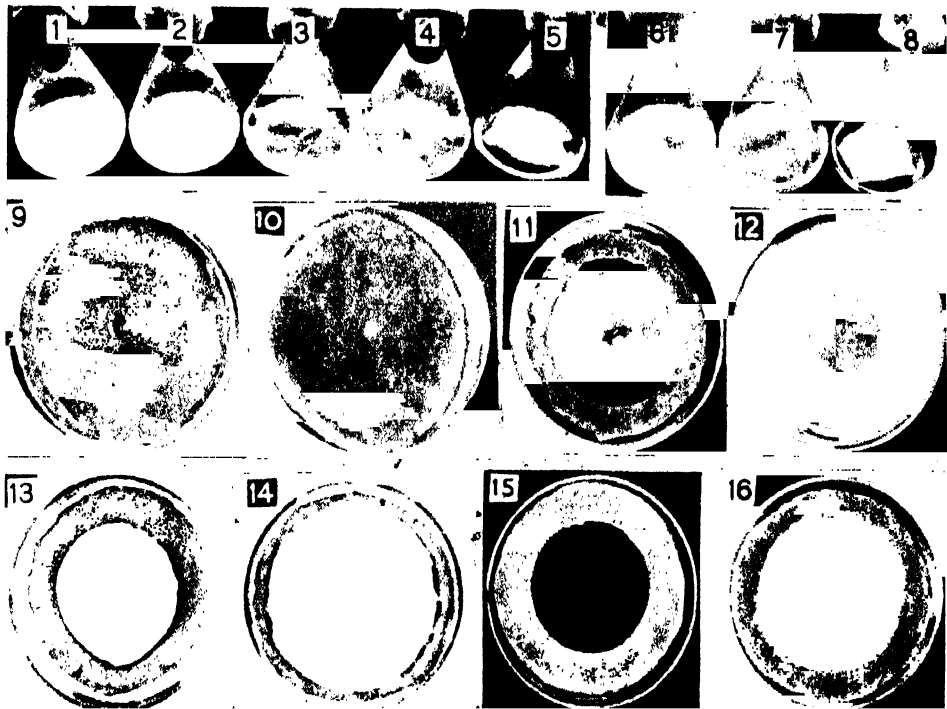
pH 5.5 seemed to be the most favourable for enzymic activity and the enzyme strength was lower when measured at higher pH levels. In contradistinction, filtrate activity of *F. dimerum*, *F. javanicum*, *F. solani* and *F. scirpi* was maximum at pH 6.5 and decreased at the lower pH levels.

On the bacterial cellulose membranes all the *Fusaria* manifested good cellulolytic activity (Text-fig. 3) with the exception of *F. caucasicum* (Plate XX, Fig. 10), which was also unable to digest filter paper. All the other species which had no cellulolytic activity on filter paper, i.e. *F. buharicum*, *F. chlamydosporum*, *F. poae* and *F. sporotrichioides*, were able to decompose the bacterial cellulose and the extent of decomposition was striking in the case of *F. chlamydosporum* and *F. sporotrichioides*, both of which made 6.2 cm. diametrical spread on the membrane (Text-fig. 3).

Out of seven species, which were poor decomposers of filter paper, only *F. semitectum* failed to make any appreciable growth on the bacterial cellulose membrane, whilst the other six species, *F. bulbigenum* var. *lycopersici*, *F. conglutinans*, *F. dimerum*, *F. equiseti*, *F. sambucinum* and *F. scirpi*, decomposed the membranes markedly. *F. udum* and *F. lateritium*, in which strongest degradation of filter paper was observed, did not make commensurate growth on the cellulose membranes, whereas *F. culmorum*, which had only moderate cellulolytic activity on filter paper, made the maximum growth on the membrane (Plate XX, Fig. 14; Text-fig. 3). *F. javanicum*, *F. lini*, *F. moniliforme*, *F. solani*, *F. orthoceras*, *F. oxysporum*, *F. oxysporum* var. *nicotianae*, *F. udum* and *F. vasinfectum* grew well on the bacterial cellulose (Text-fig. 3).

DISCUSSION

It is now well recognized that degradation of cellulose by fungi depends largely on the chemical composition of the substrate (Siu and Reese, 1953). In the experiments reported, with the exception of one species, *F. caucasicum*, all others were able to decompose bacterial cellulose. On the other hand, five species, *F. buharicum*, *F. caucasicum*, *F. chlamydosporum*, *F. poae* and *F. sporotrichioides*, were unable to digest filter paper, whilst seven others, namely *F. bulbigenum* var. *lycopersici*,



Figs. 1-8. Show the disintegration of filter paper by *Fusaria* 3 weeks after inoculation: *F. lateritium* (1), *F. sambucinum* (2), *F. lini* (3), *F. conglutinans* (4), *F. caucasicum* (5), *F. vasiofection* (6), *F. culmorum* (7) and *F. chlamydosporum* (8).

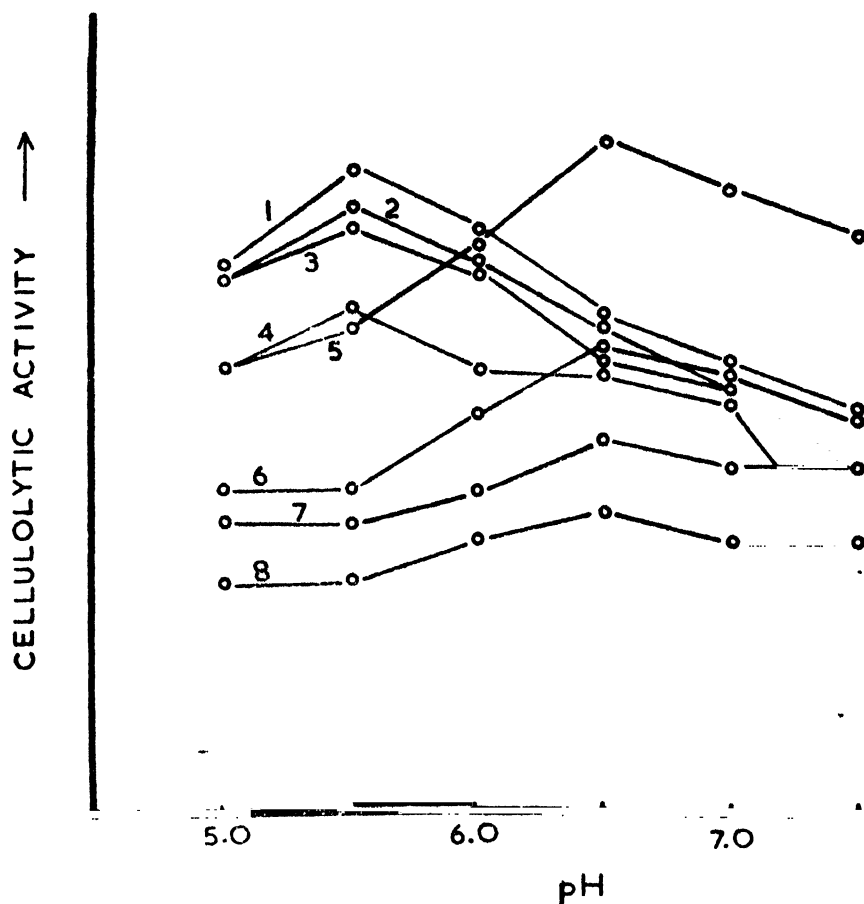
Fig. 9. Characteristic growth of *Fusarium* on bacterial cellulose membrane.

Fig. 10. Inability of *F. caucasicum* to grow on bacterial cellulose.

Fig. 11. Decomposed and undecomposed parts of the cellulose membrane separated by rubbing the surface of the *Fusarium* colony with a glass spatula.

Fig. 12. The fungal colony left behind after the removal of the undecomposed part of the cellulose membrane.

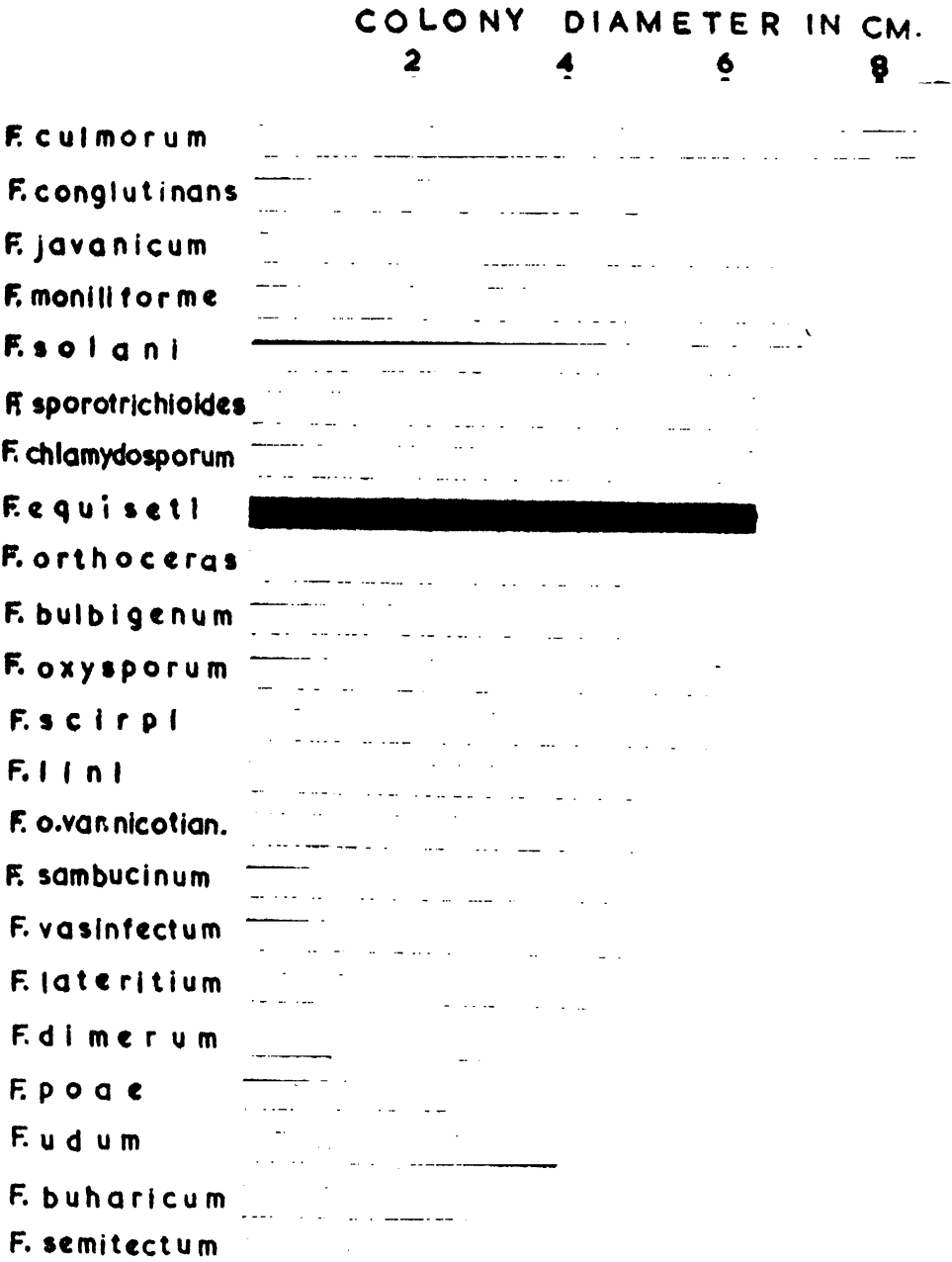
Figs. 13-16. Show the digestion of bacterial cellulose by *Fusaria*; cellulose liquefied as the result of fungal growth has been removed. *F. orthoceras* (13), *F. clunorum* (14), *F. chlamydosporum* (15), and *F. solani* (16).



TEXT-FIG. 2. Effect of pH on cellulolytic activity of *Fusarium* culture filtrates on re-precipitated filter paper; *F. moniliforme* (1), *F. lateritium* (2), *F. vasinfectum* (3), *F. lini* (4), *F. javanicum* (5), *F. solani* (6), *F. dimerum* (7) and *F. scirpi* (8).

F. conglutinans, *F. dimerum*, *F. equiseti*, *F. sambucinum*, *F. scirpi* and *F. semitectum*, were capable of only weak cellulolytic activity on this substratum. According to Siu and Reese (1953), the non-cellulosic components in the substrate determine its susceptibility to microbial decomposition. This factor may operate in two directions: one, in which the availability of the non-cellulosic constituents increases its susceptibility, and, in the other case, the resistance of the non-cellulosic ingredients to microbial attack protecting the cellulose substrate from decomposition (Basu, 1948). The inability of *F. chlamydosporum*, *F. sporotrichioides*, *F. poae* and *F. buharicum* to decompose filter paper, while causing degradation of bacterial cellulose, may have been due to either the resistant substances in the filter paper being probably toxic to these species or due to their inability to produce the specific enzyme, C_1 (Siu and Reese, 1953), which is concerned in the splitting of native cellulose. Further observations on the nature of the bacterial cellulose synthesized by *A. xylinum* which is being undertaken elsewhere (Minor *et al.*, 1954), utilizing C^{14} labelled carbon source, will no doubt throw much light on the question of the greater susceptibility of bacterial cellulose to Fusaria than filter paper.

The response of cellulase activity to pH is known to vary strikingly with different sources of enzyme. Optima for cellulolytic activity of *Aspergillus niger* and *A. oryzae* were pH 4.7 and 4.5, respectively, whereas *Myrothecium verrucaria* has an



TEXT-FIG. 3. Growth of *Fusaria* on cellulose membranes biosynthesized by *Acetobacter xylinum*, six days after inoculation.

optima around pH 5.5 (Siu, 1951). The enzymic strength of *Fusarium* culture filtrate was found to be directly related to the cellulolytic activity of the species on filter paper and also varied with the pH of the medium. Enzyme activity of filtrate of most of the species was maximum at pH 5.5 (Table I and Text-fig. 2), while filtrates of *F. dimerum*, *F. javanicum*, *F. solani* and *F. scirpi* had an optima of 6.5 (Text-fig. 2). These optima are in reference to re-precipitated cellulose and enzyme activity of the *Fusarium* filtrates is likely to vary with the nature of the

cellulose substrate used, as observed by Reese and Levinson (1952) in the case of other organisms.

It is obvious from this work that *Fusaria* manifest considerable variation in relative ability of a particular species to attack cellulose (Text-figs. 1 and 2), with the exception of *F. caucasicum*. This is in keeping with similar observations, as for example, within the genus *Aspergillus* only certain species are cellulolytic, and even within the same species of *Penicillium citrinum* only some isolates are cellulolytic (Siu and Reese, 1953). One difficulty in probing deeper into this problem has been in obtaining the fungal enzyme in a pure state; the production of these 'adaptive' enzymes only on cellulose substrates is also a limiting factor in determining the exact status of a fungal isolate in respect of cellulolytic activity and only further work in this direction could clarify the overall picture.

SUMMARY

Twenty-three species of *Fusarium* were tested for cellulolytic activity on filter paper and bacterial cellulose. Considerable variation was observed in the relative ability of a particular species to attack these two cellulose substrata. Four species, *F. buharicum*, *F. chlamydosporum*, *F. poae* and *F. sporotrichioides*, did not digest filter paper, but decomposed bacterial cellulose. Only *F. caucasicum* was inactive on both filter paper and bacterial cellulose; all the other species decomposed these cellulose substrata. Culture filtrates of *Fusaria* growing on filter paper were active on re-precipitated cellulose and the strength of the enzymic activity of the filtrate was directly related to the ability of the species to decompose filter paper. The activity of the enzyme preparation of most of the species was maximum at pH 5.5, whilst four species had an optimum of pH 6.5.

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STUDIES ON THE PHYSIOLOGY OF RICE

XII. CULTURE OF EXCISED EMBRYOS IN RELATION TO ENDOSPERM AUXIN AND OTHER GROWTH FACTORS

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INTRODUCTION

In a previous paper of this series (Sircar and Das, 1954) evidence of the presence of a large amount of auxin in the endosperm of rice and its gradual disappearance with the germination of the embryo was presented. Hatcher (1945) observed that during the development of the rye grain auxin of the endosperm mainly accumulates in aleurone cells. With ripening, however, the total auxin of the seed decreases (Avery *et al.*, 1942; Popoff, 1941 and Hatcher, 1945). Hatcher has further shown that this disappearance at maturity is due to the desiccation of the grain during ripening and not due to translocation from the grain nor any growth activity. During the early stages of germination the bound auxin is set free and the liberated auxin presumably indolyl acetic acid disappears within a few days and appears to be inactivated (Avery *et al.*, 1940 and Guttenberg *et al.*, 1947). Funke and Soding (1948) suggested that this inactivation takes place in the first internode and moves upward in the inactive form. When it reaches the coleoptile tip it becomes activated once again and moves downward in this form. According to this hypothesis indolyl acetic acid plays an important rôle in the dynamics of the early germination of embryo. Das (1954) has further shown that the embryo of the *petkus* winter rye gradually accumulates auxin with age. A similar result has been obtained by the present authors with winter rice var. *Bhasamanik*. In order to throw further light on the auxin relation of rice endosperm comprehensive experiments on the germination of rice embryo have been carried out by eliminating portions of endosperm and substituting endosperm extract and indolyl acetic acid. A brief summary of the results showing the presence of inhibitory factors in the early growth of embryo has already been published (Sircar, Das and Lahiri, 1955). The part played by the endosperm extract and added indolyl acetic acid on germination was studied by growing embryos in nutrient media. The scope of the work was extended further to analyse the effect of different growth factors on the germination of excised rice embryo.

EXPERIMENTAL METHODS

Fractioning of endosperm and germination in indolyl acetic acid and endosperm extract.—Rice grains var. *Bhasamanik* received from the State Rice Research Station, Chinsurah, West Bengal, were carefully husked so that the embryos were not damaged during husking. A mm. graph was fixed on a slide and the grain was held on it. Different fractions of endosperm were then carefully removed with the help of a sterile scalpel. The embryos with different fractions of endosperm were subsequently soaked in sterile distilled water for 24 hours in darkness at $25 \pm 10^\circ\text{C}$. They were then transferred to 1.5% sterile agar slants inside a closed culture room. No seed treatment was done to avoid the effects of other factors that might influence the growth rate of embryo. Agar tubes showing fungus contamination were immediately rejected.

In other experiments embryos with different fractions were grown in sterile agar media containing a range of conc. of indolyl acetic acid (I.A.A.) from 10 mg./L to 0.001 mg./L and endosperm extract. Endosperm extracts (0.212% and 0.0212%) were prepared according to the method described by Sircar and Das (1954). In all cases control sets with full endosperm were maintained. The seedlings in agar tubes were kept in a temperature controlled chamber ($25 \pm 1^\circ\text{C}$.) and exposed for five minutes to diffuse sunlight every day. The root and the shoot growth was measured every day by a mm. graph paper held on the tube.

Culture of excised embryo in nutrient media.—Rice embryo is usually very small (approx. 1 to 0.5 mm. in length). It was noticed that during excision any injury to the embryo or the scutellum brought about a failure of germination in most cases even when the excised embryo was placed in media containing minerals, carbohydrate, auxin and vitamins. This is presumably due to the inevitable damage to the epithelial layer during excision resulting in the disruption of translocation of nutrients to the embryo. In order to avoid any injury and damage to the epithelial layer the following procedure was adopted for these experiments. Dehusked grains were soaked in sterile distilled water for 24 hours in darkness at $25 \pm 1^\circ\text{C}$. and the excision of the embryos was made under aseptic conditions in a closed culture room. The incision was given behind the epithelial layer within 1 mm. on the endosperm tissue by a sharp arrow-headed needle under the high power of a Zeiss stereo-binocular microscope. To exhaust the food stored in the residual endosperm, embryos were germinated on sterile agar plates without nutrients. In course of 48 hours the germinating embryo used up the food of the endosperm, it grew no further and subsequently died. The 48 hours old embryos were then transferred to sterilized agar slants, one embryo per tube, containing nutrients. The excised embryos in the tubes were kept in a temperature controlled chamber at $25 \pm 1^\circ\text{C}$. in darkness. The growth readings were taken under the illumination of a red lamp fitted inside the chamber. Any tube showing slightest fungus contamination was rejected. The combination of mineral nutrients adopted by Prof. Gregory and Dr. Purvis at the Imperial College of Science, London (Das, 1954), was used in the culture of rice embryos. In addition the effects of the following growth factors were studied: Sucrose, thiamine (Vitamin B_1), pyridoxine (Vitamin B_6), nicotinic acid, indolyl acetic acid, D1-tryptophane. Media were prepared in all cases with 1.5% agar containing different growth factors. It may be noted here that experimental limitations did not permit the authors to try sub-culture for rice embryos or to continue the culture in agar tubes for longer than 144 hours on account of the large size of the seedlings.

RESULTS

Fractioning of endosperm and culture of embryo in endosperm extract and indolyl acetic acid media.—The results of embryo growth with different fractions of endosperm have been presented in Table I. Significance of the treatments was assessed by the application of *T*-test. It is apparent that the growth of root is increased when the embryo is growing with three-fourth endosperm. As regards the coleoptile growth significant difference has not been noted in all cases but root growth with three-fourth endosperm is significantly greater in all the periods.

In the medium containing endosperm extract the embryos with different fractions of endosperm show pronounced suppression of root length. In some cases embryos with three-fourth endosperm when grown in the medium containing the extract root length becomes almost equal to that of the control, i.e. embryo with full endosperm growing in the medium without added extract (Table II). This behaviour of the growth of root is clearly manifested in all the periods. No specific effect is indicated in the coleoptile growth by the addition of endosperm extract. As such the percentage of leaf break and the length of the first leaf is not much affected by the addition of endosperm extract, on the contrary embryo with one-eighth

TABLE I

Growth of rice embryos with different fractions of endosperm. Temperature $25 \pm 1^\circ\text{C}$.

Treatment	24 hours				48 hours			
	Shoot length in mm.	S.E.	Root length in mm.	S.E.	Shoot length in mm.	S.E.	Root length in mm.	S.E.
1. Embryos with full endosperm	2.961	0.2628	6.769	0.6918	10.417	2.220	34.562	2.375
2. Embryos with $\frac{3}{4}$ endosperm	3.333	0.1597	8.267	0.6935	14.000	0.651	39.961	1.338
3. Embryos with $\frac{1}{2}$ endosperm	3.300	0.2056	6.533	0.2698	12.906	0.750	31.471	1.855
4. Embryos with $\frac{1}{4}$ endosperm	2.900	0.1114	6.300	0.6572	11.533	0.895	25.961	2.117
5. Embryos with $\frac{1}{8}$ endosperm	2.500	0.2621	3.727	0.7650	4.700	0.786	7.285	2.526
6. Embryos with wounded endosperm	2.469	0.1852	9.033	0.7778	8.607	1.062	22.231	3.471

TABLE I—*contd.**Growth of rice embryos with different fractions of endosperm. Temperature $25 \pm 1^\circ\text{C}$.*

Treatment	72 hours				96 hours			
	Shoot length in mm.	S.E.	Root length in mm.	S.E.	Shoot length in mm.	S.E.	Root length in mm.	S.E.
1. Embryos with full endosperm	24.214	1.525	47.808	1.754	33.143	3.272	54.692	6.290
2. Embryos with $\frac{3}{4}$ endosperm	26.291	2.746	53.769	1.634	35.692	3.573	64.615	4.699
3. Embryos with $\frac{1}{2}$ endosperm	22.133	1.421	49.300	4.495	29.751	3.797	50.625	2.845
4. Embryos with $\frac{1}{4}$ endosperm	16.462	2.150	38.091	3.212	22.357	0.888	44.800	3.828
5. Embryos with $\frac{1}{8}$ endosperm	7.233	1.133	18.045	3.045	14.636	1.703	28.272	5.312
6. Embryos with wounded endosperm	15.143	1.529	42.393	3.682	23.250	3.617	44.000	..

endosperm shows very poor growth and coleoptile breaking does not occur up to 96 hours of culture. When the embryos with different fractions of endosperm are grown in the medium containing I.A.A. the acceleration of root growth due to elimination of endosperm fraction as noted previously is retarded and in some cases it becomes almost equal to the root length of embryos with full endosperm growing without I.A.A. (Fig. 1). A general survey from the figure will show that in all cases I.A.A. exerts a retarding effect on root growth in a definite sequence with increasing concentration. In the control set (embryos with different fractions of endosperm growing in the media without I.A.A.) the embryos with half endosperm

TABLE II

Growth of rice embryos (attached to endosperm) in different concentrations of endosperm extract. Temperature $27 \pm 1^\circ\text{C}$.

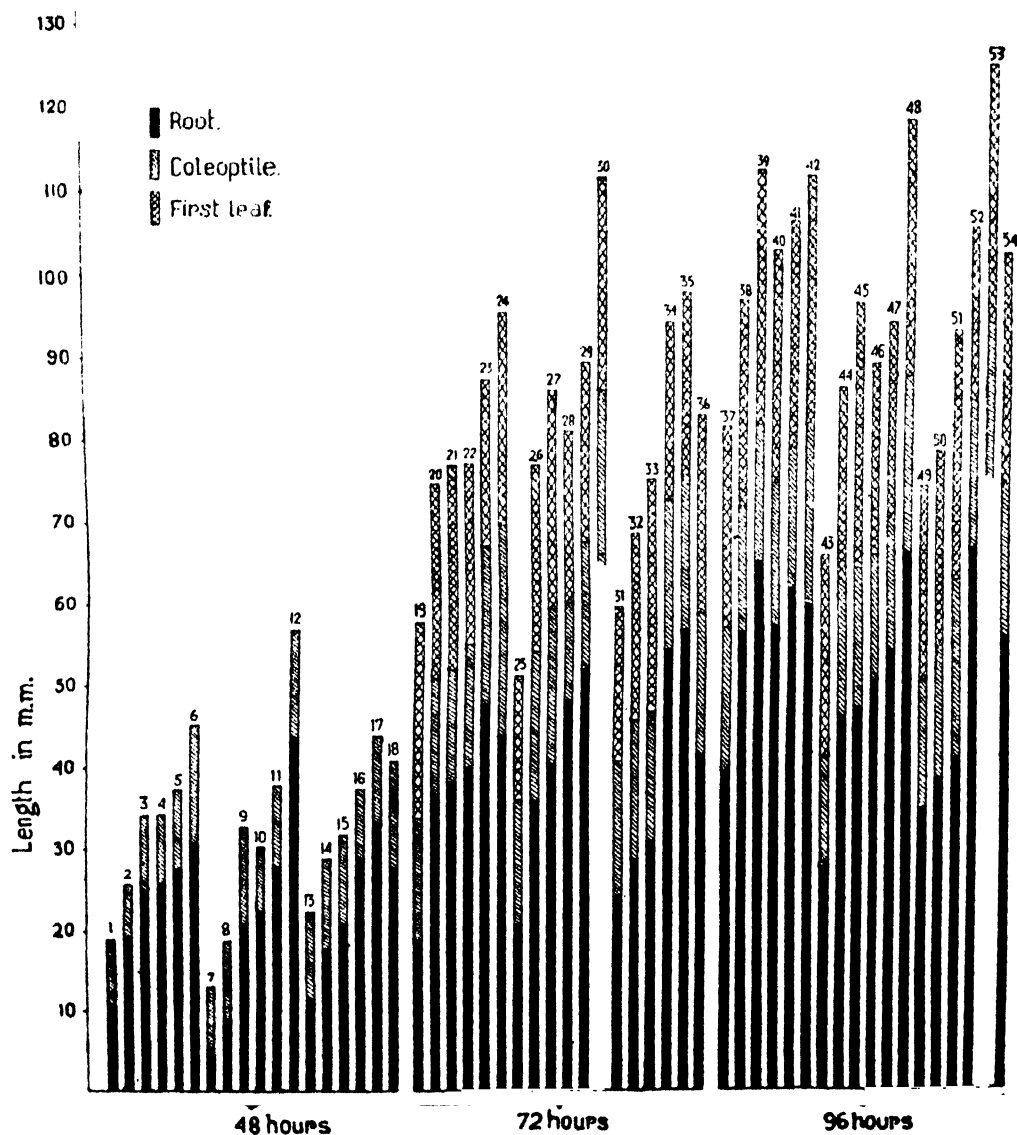
Treatment	24 hours			48 hours	
	Conc. of endosperm extract	Length of cole-optile in mm.	Length of root in mm.	Length of cole-optile in mm.	Length of root in mm.
1. Embryos with full endosperm	0.212%	1.97	4.53	5.93	22.65
	0.0212%	1.73	4.20	5.33	16.95
	Control	2.97	6.71	7.63	23.71
2. Embryos with $\frac{1}{2}$ endosperm	0.212%	1.73	4.70	4.53	18.93
	0.0212%	1.36	3.20	3.53	18.33
	Control	2.20	6.53	5.93	22.00
3. Embryos with $\frac{1}{4}$ endosperm	0.212%	1.83	3.72	6.20	19.96
	0.0212%	1.67	5.50	5.06	23.00
	Control	2.43	8.27	6.73	28.15
4. Embryos with $\frac{1}{8}$ endosperm	0.212%	1.60	2.50	3.88	6.25
	0.0212%	1.57	2.20	3.56	8.00
	Control	2.50	3.73	4.25	6.42

TABLE II—contd.

Growth of rice embryos (attached to endosperm) in different concentrations of endosperm extract. Temperature $27 \pm 1^\circ\text{C}$.

Treatment	72 hours				96 hours			
	Length of cole-optile in mm.	Length of first leaf in mm.	% of leaf break	Length of root in mm.	Length of cole-optile in mm.	Length of first leaf in mm.	% of leaf break	Length of root in mm.
1. Embryos with full endosperm	12.91	22.0	8.0	36.50	16.83	28.38	66.7	53.83
	11.71	34.62	15.79	27.27	58.3	49.91
	13.77	19.50	18.20	45.45	20.73	27.70	90.9	66.00
2. Embryos with $\frac{1}{2}$ endosperm	12.25	34.04	16.58	22.40	41.60	44.54
	11.33	39.44	13.36	20.57	46.40	50.54
	10.67	17.00	27.20	38.50	15.10	23.37	80.00	56.20
3. Embryos with $\frac{1}{4}$ endosperm	13.75	15.00	8.00	38.36	16.41	24.70	83.30	68.50
	16.11	22.00	9.00	47.64	18.33	29.42	100.00	65.33
	16.30	14.75	16.70	49.58	16.34	25.15	100.00	72.08
4. Embryos with $\frac{1}{8}$ endosperm	4.90	6.75	5.20	7.88
	5.14	8.29	6.59	8.35
	5.79	6.79	6.21	6.83

show greater root length over all other treatments. Growth of root in this particular treatment is significantly greater in all the periods. The shoot growth, however, is not much affected by the presence of I.A.A. in the culture media.



Growth of rice embryo with fractions of endosperm in media containing different concentrations of I.A.A.

FIG. 1. 48 hours.—1-5: embryos with full endosperm in 10 mg./L, 1 mg./L, 0.1 mg./L, 0.01 mg./L, 0.001 mg./L respectively of I.A.A. media and 6 is control; 7-11: embryos with $\frac{1}{2}$ endosperm in the same concentrations of I.A.A. and 12 is control; 13-17: embryos with $\frac{1}{4}$ endosperm in I.A.A. media and 18 is control.
72 hours.—19-23: embryos with full endosperm in I.A.A. media and 24 is control; 25-29: embryos with $\frac{1}{2}$ endosperm in I.A.A. media and 30 is control; 31-35: embryos with $\frac{1}{4}$ endosperm in I.A.A. media and 36 is control.
96 hours.—37-41: embryos with full endosperm in I.A.A. media and 42 is control; 43-47: embryos with $\frac{1}{2}$ endosperm in I.A.A. media and 48 is control; 49-53: embryos with $\frac{1}{4}$ endosperm in I.A.A. media and 54 is control.
The same concentration is used for different durations.

The effect of sucrose and other growth factors on excised embryos.—In order to observe the effect of sugar excised embryos are cultured in the media containing different concentrations of sucrose. In all cases mineral nutrients are added. In control no sucrose is given, only minerals are present. Without sucrose the embryos show very limited growth; when both sucrose and minerals are present maximum growth of embryo is observed at two per cent sugar (Table III).

Normally the embryos attached with the endosperm show breaking of coleoptile and initiation of first leaf on agar slants within 72 hours after sprouting and the coleoptile length usually is 1.4 to 1.5 mm. at this stage. In the culture of embryos in different concentrations of sugar no coleoptile breaking is observed even after 96 hours. In general the seedlings are very small in comparison to those remaining attached with the endosperm. When only minerals or sucrose is present in the medium the embryo growth is limited. The essential rôle of thiamine (Vitamin B₁) in plant tissue culture has been emphasized by various workers. Its presence in the pericarp of rice and the disease caused by its absence in polished rice is an established fact. Bonner (Schopfer, 1943) has shown its action is quantitative. Within certain limits any increased dosage caused an increase in growth but beyond the optimum level no effect is produced. In the present study thiamine in various concentrations is supplied with two per cent sucrose-mineral nutrients and growth rate of excised embryos are studied. It is evident from Fig. 2 that in the first 24 hours' supply

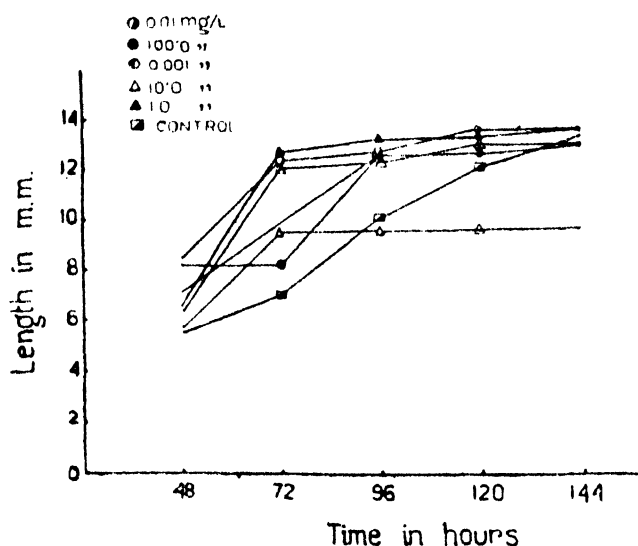


FIG. 2. Effects of different concentrations of thiamine on the coleoptile growth of excised embryos in the media containing sucrose and minerals.

of thiamine there is an increase in the growth rate up to the length of the coleoptile where it usually breaks. In the control the growth of the coleoptile is slow and it takes about 144 hours to reach this length. Coleoptile breaking and elongation of first leaf, however, did not occur up to 144 hours' growth in any of the treatments which would indicate that possibly some other factor responsible for elongation of leaf is lacking in the media. Thiamine seems to play an important rôle in the root growth of excised embryos. In its presence increase in root length takes place in almost all the concentrations. Best root growth is obtained in 0.01 mg./l. concentration (Fig. 3) which appears to be the optimum for the culture of excised rice embryo.

It has been reported that rice grains contain large amount of pyridoxine (Schopfer, 1943). Its presence in the grain suggests that it may have some rôle in

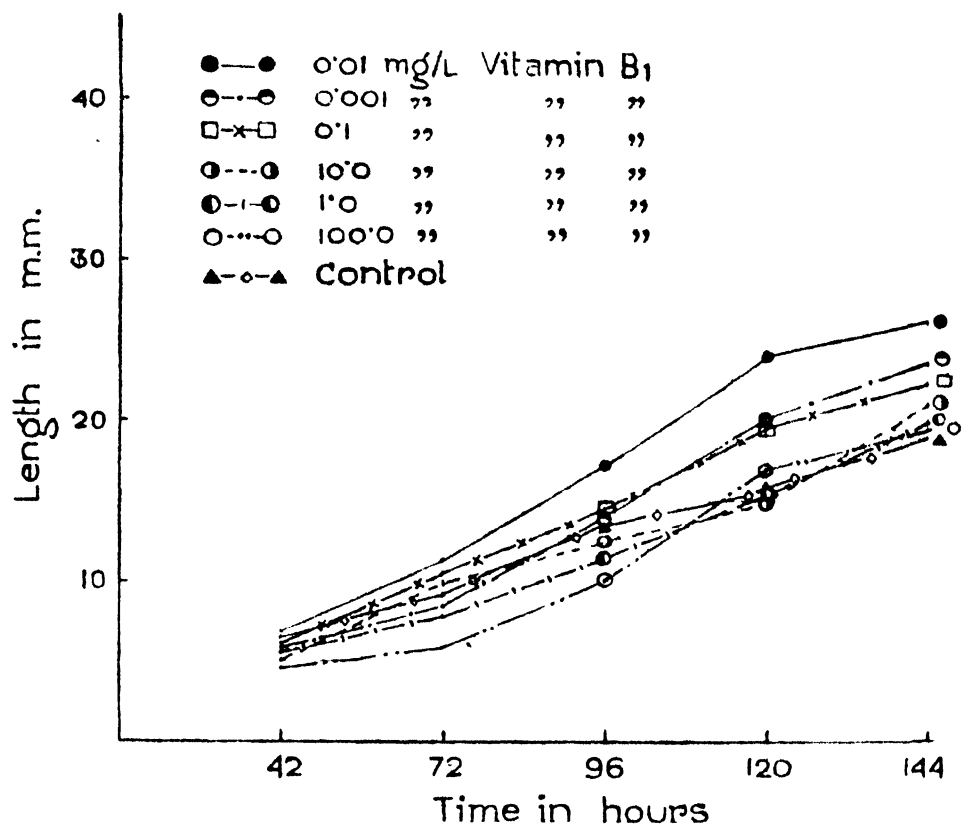


FIG. 3. Effects of different concentrations of thiamine on the root growth of excised embryos in the media of sucrose and minerals.

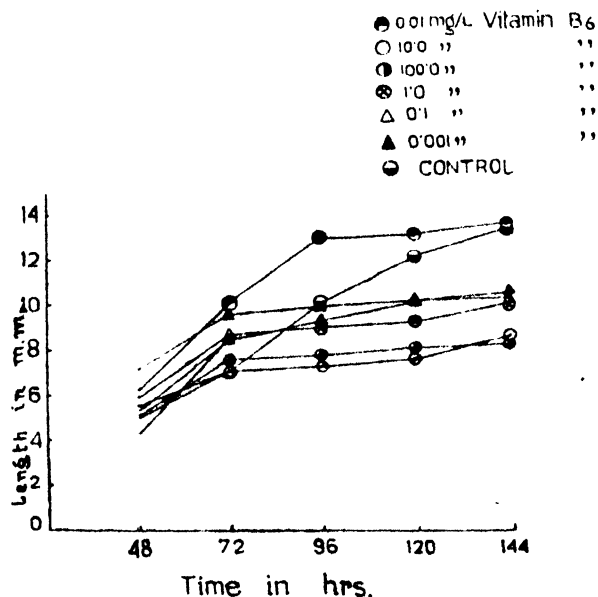


FIG. 4. Effects of different concentrations of pyridoxine on the coleoptile growth of excised embryos in the media of sucrose and minerals.

the metabolism of the growing embryo. In order to study its effect on the growth of excised rice embryo pyridoxine in combination with two per cent sucrose and minerals is used in the medium. Control set is prepared with two per cent sucrose and minerals only. Figs. 4 and 5 will show that 0.01 mg./L conc. of pyridoxine (Vitamin B₆)

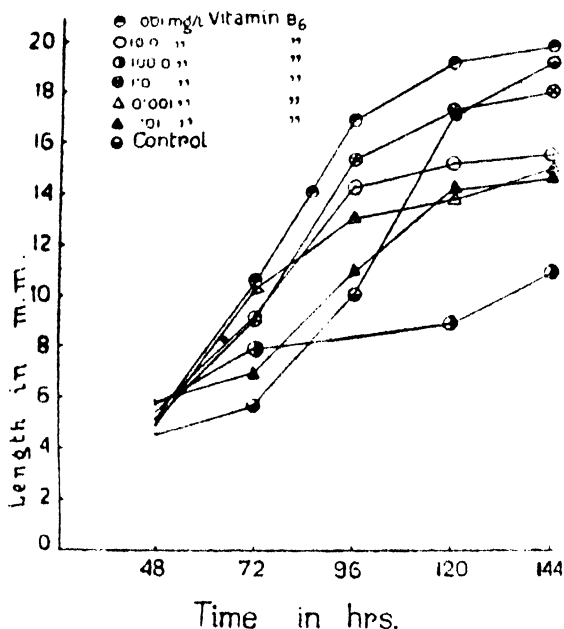


FIG. 5. Effect of pyridoxine on the root growth of excised embryos in the media containing sucrose and minerals.

increases the growth rate of root and of coleoptile at the initial stage while other concentrations do not seem to have any effect on shoot growth but root growth is slightly increased. It appears that concentrations of 10 mg./L or above have a depressing effect on root and shoot growth. In no case, however, coleoptile breaking or leaf elongation has been observed up to 144 hours.

In the next series of experiments the following combinations of growth factors have been used to study their effects on the excised embryos:—

1. Mineral nutrients + I.A.A. (0.01 mg./L) and two per cent sucrose
2. " " + I.A.A. (0.001 mg./L)
3. " " + thiamine (0.01 mg./L)
4. " " + I.A.A. (0.001 mg./L) + thiamine (0.01 mg./L) + pyridoxine (0.01 mg./L)
5. " " + I.A.A. (0.001 mg./L) + pyridoxine (0.01 mg./L) + nicotinic acid (0.01 mg./L)
6. " " + I.A.A. (0.001 mg./L) + pyridoxine (0.01 mg./L) + nicotinic acid (0.01 mg./L) + thiamine (0.01 mg./L)
7. " " + Dl-tryptophane (0.01 mg./L) + thiamine (0.01 mg./L) + pyridoxine (0.01 mg./L)
8. " " + Dl-tryptophane (0.01 mg./L) + thiamine (0.01 mg./L) + nicotinic acid (0.01 mg./L) + pyridoxine (0.01 mg./L)

A remarkable shoot growth has been observed when I.A.A. is present in the medium in combination with sucrose and minerals (treatments 1 and 2 in Fig. 6).

1. ○ Mineral nutrients + 2% sucrose + IAA (0.01 mg/L)
2. ● " " " " " + " (0.001 mg/L)
3. △ " " " " " + " " + vitamin B₁ (0.01 mg/L)
4. ▲ " " " " " + " " + " " + vit B₆ (0.01 mg/L)
- " " " " " (control)

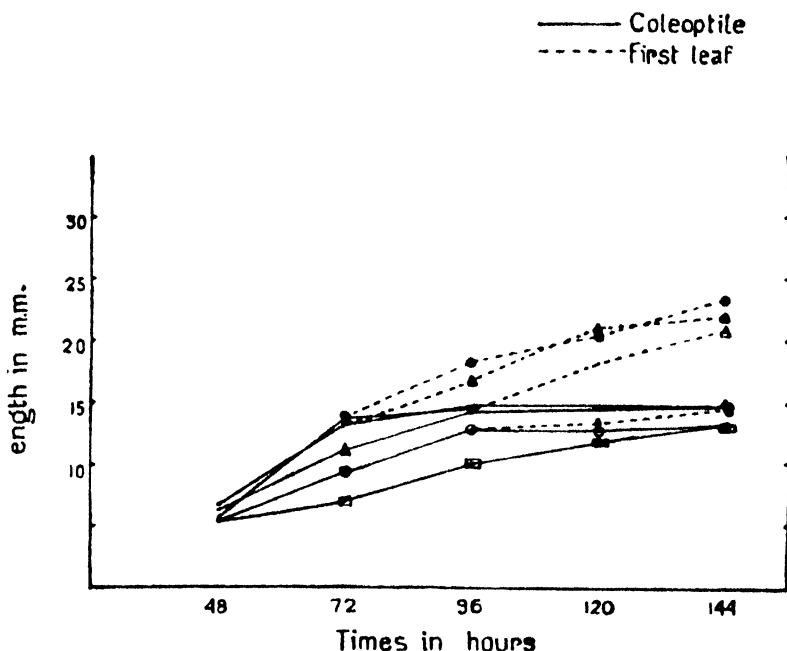


Fig. 6. Shoot growth of excised embryos in complex nutrient media.

The root growth is also accelerated on addition of I.A.A. at low concentrations (0.01 mg./L and 0.001 mg./L), growth with 0.001 mg./L being greater. Coleoptile breaking and elongation of first leaf are observed within 120 hours in treatment 2. When thiamine, pyridoxine and I.A.A. are present in the medium the embryo growth is very much favoured (treatment 4 in Figs. 6 and 7). Here root length is much greater in comparison to previous treatments. The presence of thiamine and pyridoxine together greatly favours root growth than when they are present alone. The coleoptile breaking and the elongation of first leaf is observed within 96 hours. Nicotinic acid when added with I.A.A. and pyridoxine does not show any marked improvement in growth although root and shoot development is satisfactory. Addition of thiamine in this combination does not accelerate embryo growth (treatments 5 and 6 in Figs. 8 and 9). When DL-tryptophane in combination with thiamine, pyridoxine, sucrose and minerals is used as a substitute for I.A.A. growth of both root and shoot development is accelerated (treatment 7 in Figs. 8 and 9 and Fig. 10). In addition of nicotinic acid to these factors the roots show somewhat reduced growth while the shoot development is not affected (treatment 8 in Figs. 8 and 9). It thus appears that whenever I.A.A. or DL-tryptophane is present in the medium the shoot development, i.e. coleoptile breaking and appearance of first leaf, is accelerated otherwise the coleoptile shows very limited growth.

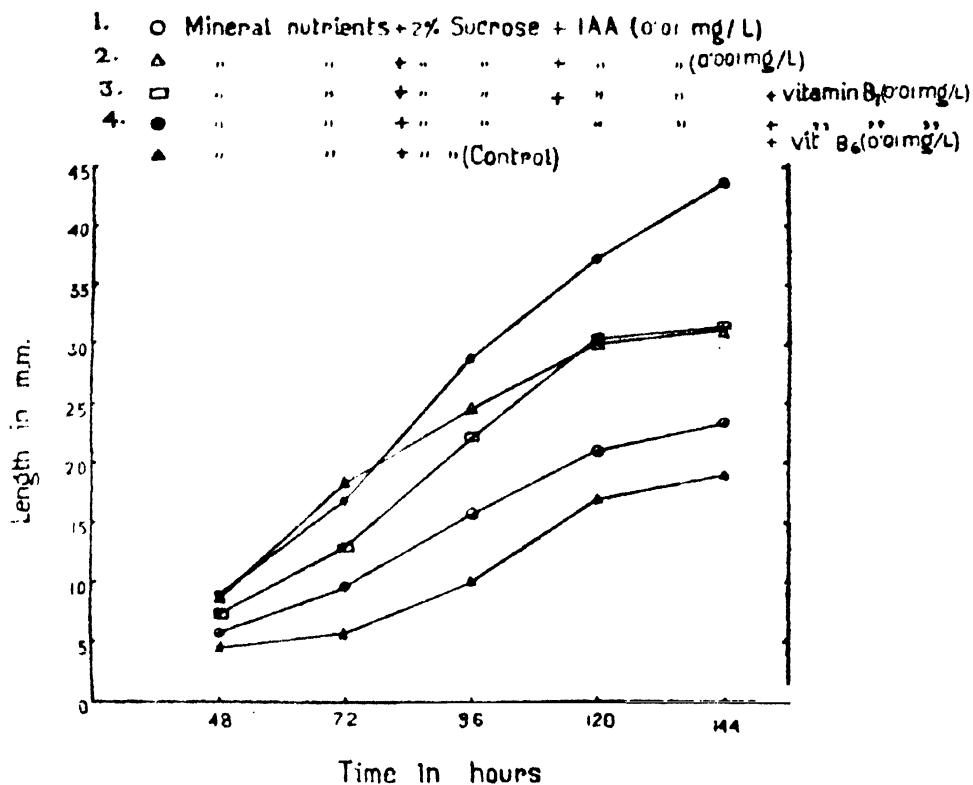


FIG. 7. Root growth of excised embryos in complex nutrient media.

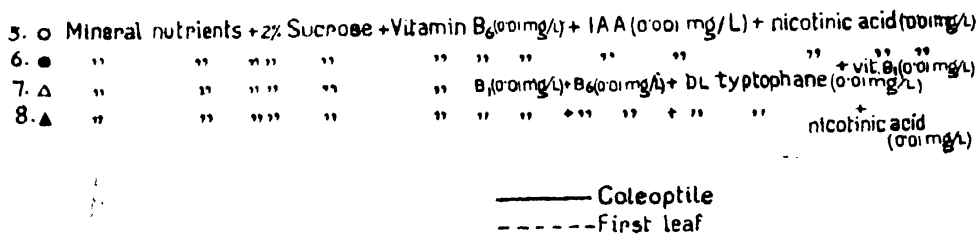


FIG. 8. Shoot growth of excised embryos in complex nutrient media.

It may be observed, again, that increased root growth by the fractioning of endosperm (i.e. half endosperm) is retarded when it is grown in the medium containing I.A.A. and it becomes almost equal to the embryo growing in agar medium (Fig. 1). The inhibition of root length caused by the presence of I.A.A. in the medium may be compared with the inhibitory factor of the endosperm which suppresses the root growth in the early stages of germination. On the basis of these findings attempts have been made to investigate the essential growth factors necessary for the embryo in the initial stages of germination. For this purpose excised embryo culture is adopted. It has been noted before that excised embryos in general show less growth in comparison with those attached to seeds. The diminished growth might be due to the following reasons:—

- (a) Mechanical injury during excision.
- (b) Limited auxin supply.
- (c) Lack of growth factors other than auxins, possibly of vitamin nature.

The effects of injury are of primary consideration in the work of embryo culture. The rice embryos fail to germinate when they are completely excised from endosperm and are placed in the media containing sugar, vitamins, and I.A.A. or endosperm extract. This is presumably due to the damage of the epithelial layer. In the present study the effect of injury has been eliminated by the adoption of a special technique of excision. By this method the epithelial layer is not injured, hence translocation of food substances from the media to the embryo is maintained. Sucrose has been proved to be the best form of carbohydrate for culturing tissues and embryos of several plants. In the post-germinal embryo culture of rice it has also been noted that the embryo growth corresponds directly with the increasing concentrations of sucrose up to two per cent level. With minerals only the embryos show very limited growth but with the addition of sucrose in the medium better results are obtained. The plants, however, are small in comparison to those that had intact endosperm. In the excised embryo culture of rice thiamine and pyridoxine have been found to be helpful for the root and shoot growth. They are known to be essential for the growth of roots. Excised roots of the majority of plants investigated are unable to synthesise them. Bonner and Bonner (1948) have shown that green leaves can synthesise thiamine and pyridoxine and these are translocated to roots. It is not known whether coleoptile tip can synthesise thiamine and pyridoxine. In order to prove conclusively that a substance is an essential factor it should be shown by experiment that not only growth is stimulated by the substance but no growth takes place in its absence. Since growth in the excised embryo of rice did not stop in absence of added thiamine or pyridoxine, rather these embryos were found to show increasing growth during the period of experimentation and there was stimulation after addition of these factors, it leads one to consider the importance of thiamine and pyridoxine for the growth of rice embryo. There may be two possibilities, either the coleoptile and root tips might have carried them from the endosperm during the period of residual germination or the tips are capable of synthesising them from sucrose and nutrients.

Presence of I.A.A. facilitates the shoot development to a great extent while root growth is found to be more dependent on growth factors of vitamin nature. When I.A.A. is added to the medium in combination with thiamine, pyridoxine, sucrose and minerals the optimal growth of embryo results. Tryptophane facilitates the embryo growth. The capacity of plant tissue to form auxin from tryptophane has been demonstrated by a number of workers. Such conversion probably takes place in the rice embryos in the media containing tryptophane.

It should be noted, however, that in all the different media that have been used in the present study the excised embryos show less growth in comparison to that obtained with the intact endosperm. Presumably some other unknown growth factor or factors are present which have not been included in the present investigation.

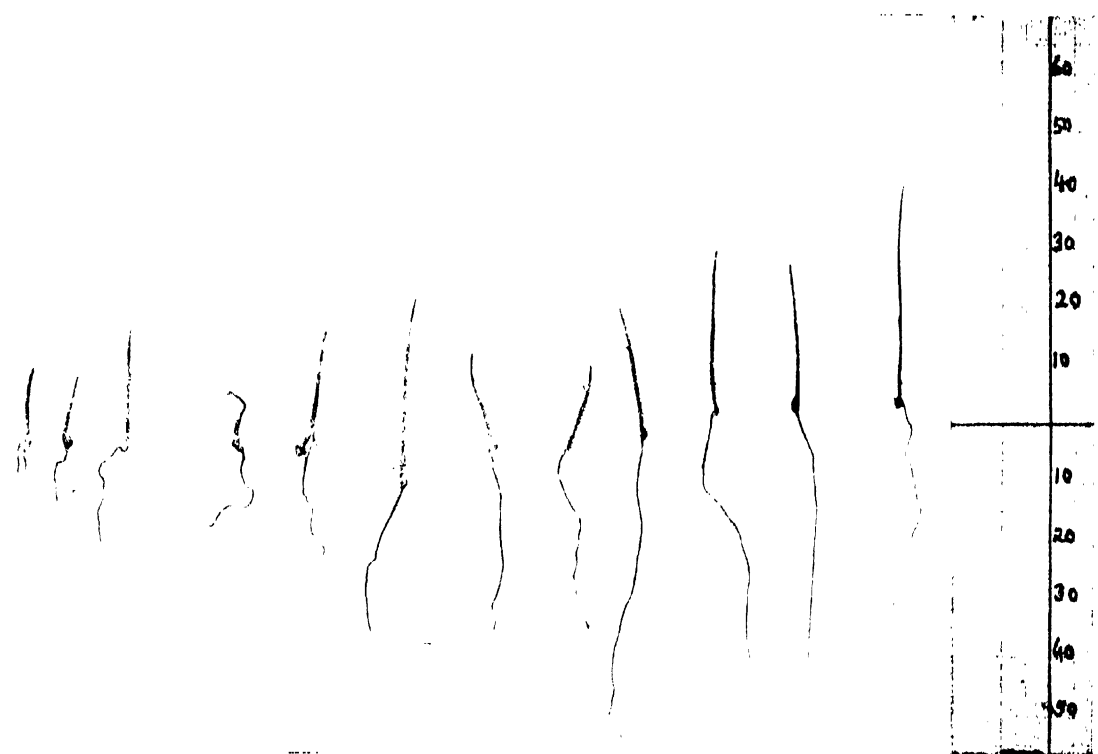


FIG 10. Photograph showing the growth and coleoptile breaking of excised embryos at different stages in the media containing 0.01 mg./L. thiamine and 0.01 mg./L. pyridoxine and DL-tryptophane media. In all cases thin root system, absence of visible root hairs and delicate growth of embryos are remarkable.

SUMMARY

Experiments on the germination of rice embryo have been carried out by eliminating fractions of endosperm and substituting endosperm extract and indolyl acetic acid.

Some factor or factors are present in the endosperm in supra-optimal concentration which exerts a retarding effect on the embryo growth in the initial stages of germination. Elimination of a fraction of endosperm makes the level of the factor optimal for embryo growth until such elimination reaches a limiting value. That this unknown factor is of auxin in nature has been shown from substitution of endosperm extract and I.A.A. in the culture media.

Attempts to culture rice embryo have shown that it fails to germinate when completely excised from the endosperm and placed in nutrient media. This was due to the damage of the epithelial layer during excision and could be eliminated by adopting a special technique.

Culture of excised embryo has shown its growth is dependent on endosperm food factors. Some of these factors are sucrose, salts, I.A.A. and vitamins B₁ and B₆. DL-tryptophane acts as a substitute for I.A.A. for the growth of embryo showing its conversion to I.A.A. in the embryo cells.

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STUDIES IN THE AMINO ACID COMPOSITION OF *FUSARIUM* MYCELIUM

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INTRODUCTION

With the advent of the toxin theory of wilting in tomato caused by *Fusarium lycopersici* (Gäumann, 1951), much attention has been focussed on the production of these toxins both *in vitro* and *in vivo* (Gäumann *et al.*, 1952, 1953; Fluck and Richle, 1955; Sanwal, 1955; Lakshminarayanan and Subramanian, 1955; Kalyanasundaram and Venkata Ram, 1956). Recently, Fluck and Richle (1955) from chromatographic studies of amino acid metabolism of *F. lycopersici* concluded that certain amino acids (alanine and citrullin) might be the precursors of the phyto-toxin fusaric acid produced by the pathogen. These observations have been further strengthened by the results obtained by Sanwal (1955).

Application of the paper partition chromatographic technique for the detection of amino acids, sugars, vitamins, etc., is being made widely in biological sciences and has been recently utilized in taxonomical studies (Bidwell *et al.*, 1952; Buzzati-Traverso and Rehnitz, 1953; Mansford and Raper, 1956). In the present investigation the free and bound amino acids of *Fusarium* mycelium of twenty-two species were studied to determine whether any association existed between the formation of these acids and the taxonomic position of these species. This work was also carried out to study the amino acids synthesized by *Fusaria* as a possible guide to their ability to produce toxins from these amino acid precursors.

MATERIALS AND METHODS

Biological material.—*Fusaria* were obtained from the Centraalbureau voor Schimmelcultures, Baarn, Holland, and from these monoconidial cultures were made and maintained on potato-dextrose agar for at least ten cultural generations prior to being employed for investigation. The species selected for investigation represented nine different taxonomic groups (Wollenweber and Reinking, 1935): *F. buharicum*, *F. culmorum* and *F. sambucinum*, section *Discolor*; *F. causicum*, *F. javanicum* and *F. solani* (*Martiella*); *F. lateritium* (*Lateritium*); *F. bulbigenum* var. *lycopersici*, *F. conglutinans*, *F. lini*, *F. orthoceras*, *F. oxysporum*, *F. udum* and *F. vasinfectum* (section *Elegans*); *F. chlamydosporum*, *F. poae* and *F. sporotrichioides* (*Sporotrichiella*); *F. equiseti* and *F. scirpi* (*Gibbosum*); *F. dimerum* (*Eupionnotes*); *F. semitectum* (*Arthrosporiella*); *F. moniliforme* (*Liseola*).

Culture methods.—Mycelium inoculum was obtained from cultures grown on Richard's medium for two weeks at 25–29°C. Further incubation resulted in onset of autolysis in many species accompanied by decrease of free amino acids present.

Culture extraction.—The fungal mycelium was extracted with 70 per cent ethyl alcohol at room temperature (25–29°C.) for 24 hours, centrifuged and the clear supernatant layers were then concentrated *in vacuo* to dryness. Chromatographic separation of the amino acids in the extract made with 70 per cent ethanol was found to be very unsatisfactory due to interference by other salts and therefore desalting of the extract was found necessary. This was achieved with good results

by extracting the amino acids in 5 ml. of a mixture containing 6 parts of *n*-butanol and 4 parts of phenol as advocated by Verghese (1956).

Determinations of the bound amino acids were made on the cellular material which was previously extracted with 70 per cent ethanol. The material was dried in desiccator *in vacuo*, ground to a fine powder and 500 mg. of the cellular material of each of the species were hydrolyzed by boiling for 24 hours in 35 ml. of 6 N HCl under a reflux condenser. The hydrolyzate was then concentrated to 2-3 ml. volume under reduced pressure at 70°C. and kept in a desiccator over KOH *in vacuo* to remove the last traces of HCl. The amino acids in the hydrolyzate were extracted with 5 ml. of the *n*-butanol phenol mixture for chromatography.

Culture analysis.—The extracts containing the free and bound amino acids were analyzed by paper partition chromatography (Consden, Gordon and Martin, 1944), employing essentially the uni- and 2-dimensional chromatographic methods (Block *et al.*, 1952). Chromatograms were run on Whatman No. 1 filter paper employing *n*-butanol:acetic acid:water (4:1:5) and phenol:water (3:1) as solvents for the first and the second dimensions, respectively. After development, chromatograms were first air dried, sprayed with 0.2 per cent ninhydrin in acetone and heated at 65°C. for 20-30 minutes to increase the intensity of the colour spots. The identity of the spots was established by comparing the R_f values of the amino acids in a known mixture with those in the extract, by the characteristic colour of the spots and by superimposing known amino acids over spots of the experimental material prior to irrigating the chromatograms.

Visual comparison of the chromatograms was made based on the fact that the size and colour intensity of the spots is largely a function of the concentrations of the compounds (Dent, 1948), and the relative amounts of the amino acids present are given on the basis of such comparisons.

RESULTS

The free amino acids detected in the mycelium of the *Fusarium* species are shown in Table I. The following free amino acids were found in the mycelial extract of all the species: aspartic acid, glutamic acid, threonine, alanine, glutamine, lysine, arginine, valine, phenylalanine and tyrosine. In addition, the culture extracts of all the species, excepting *F. equiseti*, *F. scirpi* and *F. dimerum*, contained serine and glycine, whilst proline was detected in the mycelium of *F. javanicum*, *F. conglutinans*, *F. orthoceras*, *F. lini*, *F. lycopersici* and *F. udum*. With the exception of *F. chlamydosporum*, *F. sporotrichioides*, *F. equiseti*, *F. scirpi*, *F. dimerum* and *F. semitectum*, all the other extracts contained cystine.

Gamma-amino butyric acid was detected in the extract of *F. moniliforme* (Fig. 1), *F. vasinfectum*, *F. chlamydosporum* and *F. sporotrichioides*. An unknown substance giving a yellow colour after ninhydrin spray, spot 16 (Fig. 1), was detected in the extracts of *F. moniliforme*, *F. sambucinum*, *F. lateritium*, *F. vasinfectum*, *F. sporotrichioides*, *F. equiseti* and *F. scirpi*. Another unknown, spot 17 (Fig. 1), was present in the chromatograms of *F. moniliforme*, *F. vasinfectum* and *F. sporotrichioides*.

Quantitative differences in the amino acid content in the free form in the mycelium were apparent even within species belonging to the same taxonomic group viz., in *F. javanicum* and *F. solani* (section Gibbosum) (Table I.), although the overall amino acid composition was similar. Marked quantitative as well as qualitative dissimilarities in the amino acids present in the mycelium of *Fusaria* belonging to different taxonomic groups were observed (Table I).

The bound amino acids found in the culture of the *Fusarium* species (Table II) were: aspartic acid, glutamic acid, serine, glycine, threonine, alanine, lysine, arginine, proline, valine, leucine and/or isoleucine (with the exception of *F. oxysporum*), phenylalanine and tyrosine (excepting *F. sambucinum* and *F. chlamydosporum*).

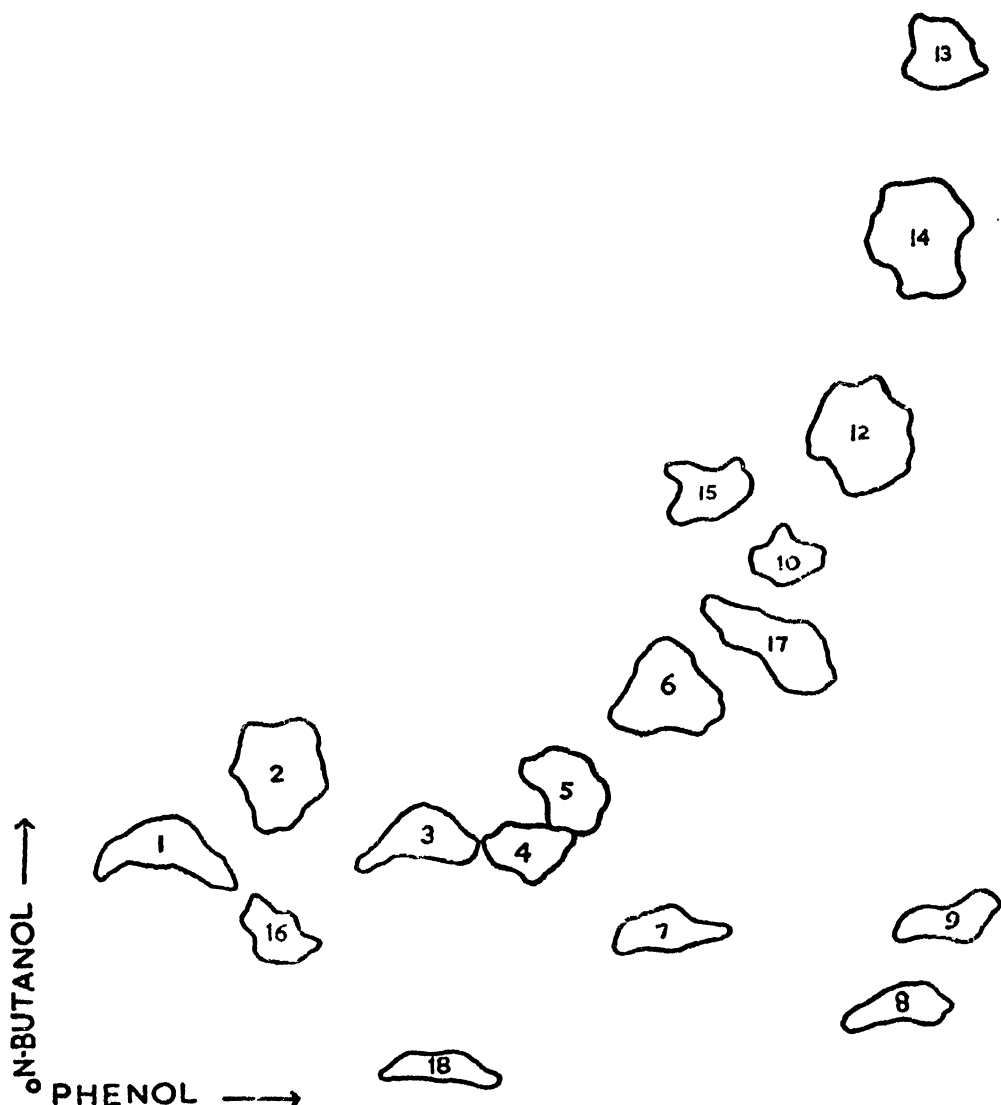


FIG. 1. Free amino acids occurring in the mycelium of *Fusarium moniliforme*. [Identity of spots in Figs. 1 & 2: (1) Aspartic acid, (2) Glutamic acid, (3) Serine, (4) Glycine, (5) Threonine, (6) Alanine, (7) Glutamine, (8) Lysine, (9) Arginine, (10) Gamma-amino butyric acid, (11) Proline, (12) Valine, (13) Leucine and/or isoleucine, (14) Phenyl-alanine, (15) Tyrosine, (16) Unknown, (17) Unknown, (18) Cystine].

Spot 10 in the chromatogram of *F. sambucinum*, *F. culmorum* (Fig. 2) and *F. buharicum* corresponded in position to gamma-amino butyric acid, while another unknown, spot 16, which gave a yellow colour with ninhydrin, was present in the extracts of *F. culmorum* (Fig. 2), *F. solani*, *F. javanicum*, *F. lateritium*, *F. equiseti* and *F. scirpi*. Differences in the chromatographic pattern of bound amino acids in mycelium of *Fusaria* belonging to different as well as the same taxonomic group seemed to be more quantitative than qualitative (Fig. 3, Table II).

Simonart and Chow (1954) stated that the amino acid composition of the mycelium of *Aspergillus oryzae* was considerably influenced by the carbon source in the substrate. To test this in *Fusaria*, mycelium of *F. moniliforme* harvested

TABLE I
Showing the free amino acids present in the mycelium of *Fusaria*

<i>Fusarium</i> spp.	Aspartic acid	Glutamic acid	Serine	Glycine	Threonine	Alanine	Glutamine	Lysine	Arginine	(gamma-amino butyric acid)	Proline	Valine	Leucine and/or isoleucine	Phenylalanine	Tyrosine	Cystine	Unknown†	Unknown‡
<i>F. sambucinum</i>	* x	x	x	x	x	xx	x	x	x			x	x	x	x	x	x	x
<i>F. culmorum</i>	x	x	x	x	x	xx	x	x	x			x	x	x	x	x	x	x
<i>F. buharicum</i>	x	x	x	x	x	xx	x	x	x			x	x	x	x	x	x	x
<i>F. solani</i>	x	x	x	x	x	xx	x	x	x			x	x	x	x	x	x	x
<i>F. caucasicum</i>	xx	xx	x	x	x	x	x	x	x			x	x	x	x	x	x	x
<i>F. javanicum</i>	x	x	x	x	x	xx	x	x	x			x	x	x	x	x	x	x
<i>F. lateritium</i>	xx	xx	x	x	x	xx	x	x	x			x	x	x	x	x	x	x
<i>F. orthoceras</i>	x	x	x	x	x	xx	x	x	x			x	x	x	x	x	x	x
<i>F. oxysporum</i>	x	x	x	x	x	xx	x	x	x			x	x	x	x	x	x	x
<i>F. conglutinans</i>	x	x	x	x	x	xx	x	x	x			x	x	x	x	x	x	x
<i>F. lini</i>	x	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x
<i>F. bulbigenum</i> var. <i>lycopersici</i>	x	x	x	x	x	xx	x	x	x			x	x	x	x	x	x	x
<i>F. udum</i>	x	x	x	x	x	xx	x	x	x			x	x	x	x	x	x	x
<i>F. vasinfectum</i>	x	x	x	x	x	xx	x	x	x			x	x	x	x	x	x	x
<i>F. sporotrichoides</i>	x	x	x	x	x	xx	x	x	x			x	x	x	x	x	x	x
<i>F. chlamydosporum</i>	x	x	x	x	x	xx	x	x	x			x	x	x	x	x	x	x
<i>F. poae</i>	x	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x
<i>F. equiseti</i>	x	x	x	x	x	xx	x	x	x			x	x	x	x	x	x	x
<i>F. scirpi</i>	x	x	x	x	x	xx	x	x	x			x	x	x	x	x	x	x
<i>F. dimerum</i>	x	x	x	x	x	xx	x	x	x			x	x	x	x	x	x	x
<i>F. semitectum</i>	x	xx	x	x	x	xx	x	x	x			x	x	x	x	x	x	x
<i>F. moniliforme</i>	x	x	x	x	x	xx	x	x	x			x	x	x	x	x	x	x

* The number of x signs indicate the relative amounts of each amino acid.

† Spot 16 in Fig. 1 which gave a yellow colour with ninhydrin.

‡ Spot 17 in Fig. 1 which gave a purple colour with ninhydrin.

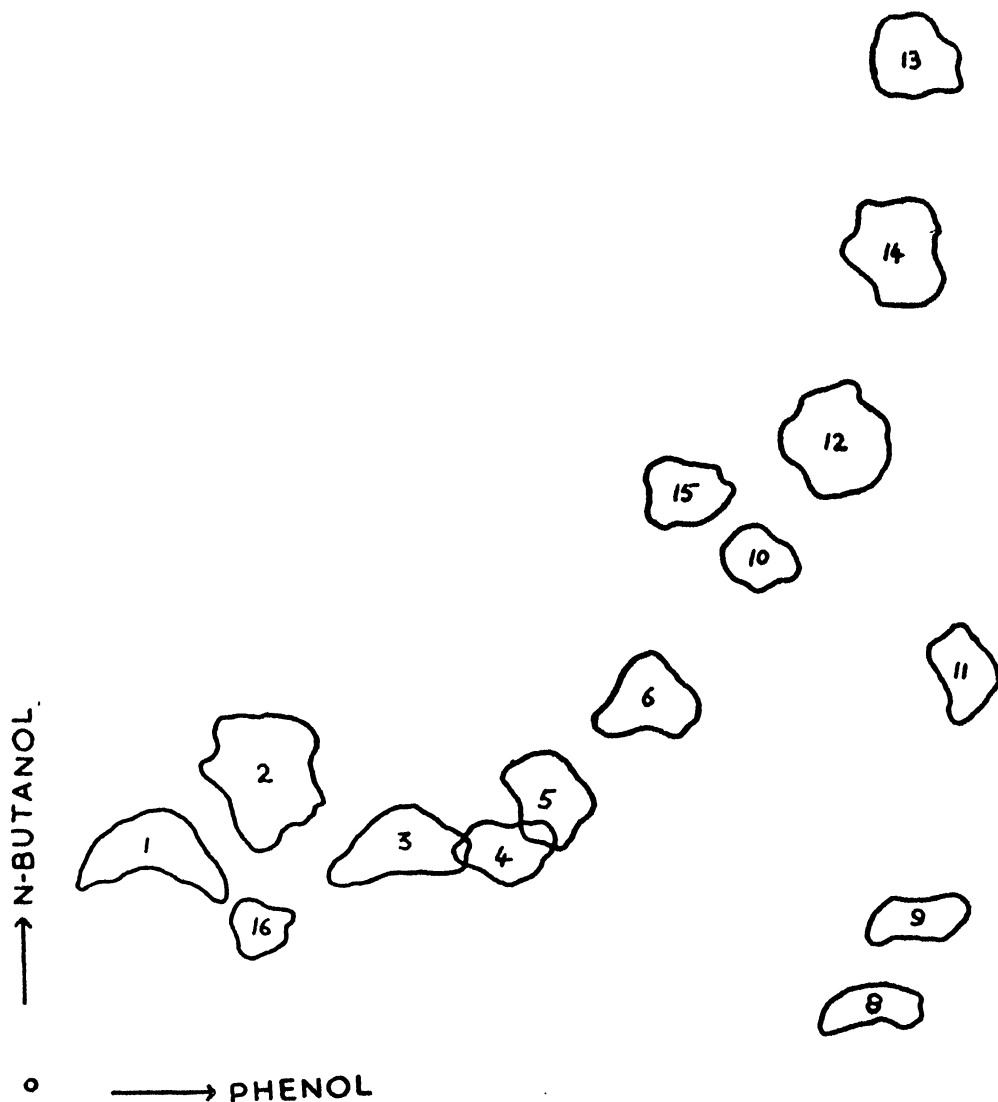


FIG. 2. Bound amino acids occurring in the mycelium of *F. culmorum*.

from a two week-old culture grown on Richard's medium containing 50 g./l. arabinose, glucose, fructose, starch and sucrose was examined for free amino acids present (Table III). Gamma-amino butyric acid was detected in extracts of mycelium harvested from sucrose and fructose cultures only, while threonine and tyrosine were found in extracts of glucose, fructose and sucrose cultures only. Two unknown ninhydrin positive substances were detected in extracts of glucose and sucrose and not in the other cultures.

DISCUSSION

Earlier work of Fluck and Richle (1955) and Sanwal (1955) indicated the possibility of the production of the phytotoxin fusaric acid from certain amino acids synthesized by *F. lycopersici*, particularly alanine and citrullin. In the present investigation on the amino acids synthesized by twenty-two species of *Fusaria*, alanine was found to occur in free and bound forms in all the species. Of these

TABLE II
Showing the bound amino acids present in the mycelium of *Fusaria*

<i>Fusarium</i> spp.	Aspartic acid	Glutamic acid	Serine	Glycine	Threonine	Alanine	Lysine	Arginine	Gamma-amino butyric acid	Proline	Valine	Leucine and/or isoleucine	Phenylalanine	Tyrosine	Unknown*
<i>F. sambucinum</i>	++	xx	x	x	xx	xx	x	x	xx	x	x	x	xxx	xx	x
<i>F. culmorum</i>	xx	xx	x	x	xx	xx	x	x	xx	x	x	xx	xxx	xx	x
<i>F. bulbaricum</i>	xx	xx	x	x	xx	xx	x	x	xx	x	x	xx	xxx	xx	x
<i>F. solani</i>	xx	xx	x	x	xx	xx	x	x	xx	x	x	xx	xxx	xx	x
<i>F. caucasicum</i>	xx	xx	x	x	xx	xx	x	x	xx	x	x	xx	xxx	xx	x
<i>F. javanicum</i>	xx	xx	x	x	xx	xx	x	x	xx	x	x	xx	xxx	xx	x
<i>F. lateritium</i>	xx	xx	x	x	xx	xx	x	x	xx	x	x	xx	xxx	xx	x
<i>F. orthoceras</i>	xx	xx	x	x	xx	xx	x	x	xx	x	x	xx	xxx	xx	x
<i>F. oxysporum</i>	x	xx	x	x	xx	xx	x	x	xx	x	x	xx	xxx	xx	x
<i>F. conglutinans</i>	xx	xx	x	x	xx	xx	x	x	xx	x	x	xx	xxx	xx	x
<i>F. lini</i>	xx	xx	x	x	xx	xx	x	x	xx	x	x	xx	xxx	xx	x
<i>F. bulbigenum</i> var. <i>lycopersici</i>	xx	xx	x	x	xx	xx	x	x	xx	x	x	xx	xxx	xx	x
<i>F. udum</i>	xx	xx	x	x	xx	xx	x	x	xx	x	x	xx	xxx	xx	x
<i>F. vasinfectum</i>	xx	xx	x	x	xx	xx	x	x	xx	x	x	xx	xxx	xx	x
<i>F. sporotrichoides</i>	xx	xx	x	x	xx	xx	x	x	xx	x	x	xx	xxx	xx	x
<i>F. chlamydosporum</i>	xx	xx	x	x	xx	xx	x	x	xx	x	x	xx	xxx	xx	x
<i>F. poae</i>	xx	xx	x	x	xx	xx	x	x	xx	x	x	xx	xxx	xx	x
<i>F. equiseti</i>	xx	xx	x	x	xx	xx	x	x	xx	x	x	xx	xxx	xx	x
<i>F. scirpi</i>	xx	xx	x	x	xx	xx	x	x	xx	x	x	xx	xxx	xx	x
<i>F. dimerum</i>	xx	xx	x	x	xx	xx	x	x	xx	x	x	xx	xxx	xx	x
<i>F. semitectum</i>	xx	xx	x	x	xx	xx	x	x	xx	x	x	xx	xxx	xx	x
<i>F. moniliforme</i>	xx	xx	x	x	xx	xx	x	x	xx	x	x	xx	xxx	xx	x

* Spot 16 in Fig. 4 which gave a yellow colour with ninhydrin.

† The number of x signs indicate the relative amounts of each amino acid.

studied, the ability to synthesize fusaric acid *in vitro* has been reported only in *F. vasinfectum*, *Gibberella fujikuroi* (*F. moniliforme*), *F. lycopersici* and *F. orthoceras* (Venkata Ram, 1956). Plausibly, the other species investigated are unable to convert the alanine and other amino acid precursors into fusaric acid through the absence of specific enzyme systems concerned in the process. The production of

TABLE III
Showing the amino acids (free) detected in the mycelium of *F. moniliforme* grown on various carbon sources

	Aspartic acid	Glutamic acid	Serine	Glycine	Threonine	Alanine	Lysine	Arginine	(gamma-amino butyric acid)	Glutamine	Valine	Leucine and/or isoleucine	Phenylalanine	Tyrosine	Cystine	Unknown*	Unknown†
Arabinose	xx	xx	x	x	x	xxx	x	x	x	x	x	x	xx	x	x	x	x
Glucose	xx	xx	x	x	x	x	x	x	x	x	x	x	xx	x	x	x	x
Fructose	xx	xx	x	x	x	xxx	x	x	x	x	x	x	xx	x	x	x	x
Starch	xx	xx	x	x	x	xx	x	x	x	x	x	x	xx	x	x	x	x
Sucrose	xx	xx	x	x	x	xx	x	x	x	x	x	x	xx	x	x	x	x

* Gave a yellow colour with ninhydrin.

† Gave a purple colour with ninhydrin.

fusaric acid, therefore, seems to bear little relationship with the amino acid synthesis in *Fusaria*.

Chromatographic studies of amino acids and other constituents of plant and animal species undertaken from a taxonomic point of view have yielded largely negative results. However, Work and Dewey (1953) examining the chromatograms of hydrolyzates of 118 micro-organisms found that the presence of α , ϵ -diaminopimelic acid was specific to certain taxonomic groups of bacteria and algae only. Similarly, Buzzati-Traverso and Rechnitzer (1953) were able to obtain a positive correlation between amino acid content and the taxonomic position of certain fishes.

DeVay (1954) working with *Ustilago zeae* failed to obtain any correlation between amino acids synthesized in culture and their sex and pathogenicity, whilst Murray and Zscheile (1956) showed that amino acid synthesis is not a limiting factor in chlamydospore production in *Tilletia caries*. Similarly, it was observed here that no relationship existed between amino acid content and the taxonomic position of a particular *Fusarium* species. Indeed, variations both quantitative as well as qualitative with free and bound amino acid pattern of the fungal mycelium were as much between species of different taxonomic groups as in species within the same group. These results are in line with the very recent findings of Mansford and Raper (1956) who demonstrated that no correlation existed between the taxonomic position and the amino acid content of many of the higher and lower plant forms studied by them.

As shown in Table III, there is evidence to indicate that the ability to synthesize amino acids in culture is considerably influenced by the carbon source present in the substrate, thus confirming the observations previously reported in *Aspergillus oryzae* (Simonart and Chow, 1954).

SUMMARY

Quantitative and qualitative differences were found in the free amino acid content of cultures of twenty-two species of *Fusarium* but were not correlated with the taxonomic position of the species. Only slight quantitative differences were found in the bound amino acid content of the different species tested. Fifteen known amino acids and one unknown ninhydrin positive substance were identified as occurring both in the bound and free forms in the *Fusarium* mycelium; in addition, another unknown substance was detected in the free form. Qualitative changes in the carbon sources present in the substrate considerably influenced the free amino acid composition of the mycelium of *F. moniliforme*. *Fusarium* mycelium harvested from a two week-old culture in Richard's medium contained aspartic acid, glutamic acid, serine, glycine, threonine, alanine, glutamine, lysine, arginine, valine, leucine and/or isoleucine, phenylalanine, tyrosine in the free form in the majority of the species, whilst in addition to these and with the exception of glutamine, proline was always detected in the bound form. Gamma-amino butyric acid was found to occur in the free and bound form in certain species.

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FIG. 3. Unidimensional chromatogram of bound amino acids detected in the mycelium of (1) *F. sporotrichoides*, (2) *F. chlamydosporum*, (3) *F. poae*, (4) *F. equiseti*, (5) *F. scirpi*, (6) *F. semitectum*, (7) *F. dimerum*, (8) *F. moniliforme*.

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NODAL ANATOMY AND THE VASCULAR SYSTEM OF THE SHOOT OF RICE PLANT (*ORYZA SATIVA* L.)

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INTRODUCTION

The rice plant is an annual grass. Its culm is erect, cylindrical and smooth, slightly swollen at the node which is solid but hollow in the internode. The leaves are distichous, base of each ligulate leaf forms a tube (sheath) completely enclosing the whole of the upper internode but split down the side opposite the blade except at the node of its insertion.

The courses of the vascular bundles through the internodes, nodes and leaf bases, i.e. the vascular system of the shoot of grasses, have rarely been studied in detail, and our knowledge in this respect is rather incomplete. De Bary (1884) described the structure of the grass node as a 'complex and confused felt of transverse bundles in each node' (pp. 262, 264 and 311). Haberlandt (1914) coming long after stated that 'the true form of the vascular system is obscured owing to the great elongation of the internode (20-50 or more) . . . and are connected at the nodes by an abundantly branched network of transverse anastomoses' (p. 384).

Bugnon (1920a) studied the origin and nature of the transverse vascular system that form the nodal network or plexus (*lacs aux noeuds*) in the apical buds of the seedlings of *Oryza sativa*. According to him such a system is formed by the sudden change of direction of the longitudinally running leaf-trace bundles on entering the node from the leaf-sheath, though Mohl attributed its independent origin to the activity of a newly born (*naissant*) secondary meristem within the nodal diaphragm. In a second paper (1920b) on the subject he tried to explain the causes of such transverse journey (*causes du parcours*) of the leaf-traces in the grass node.

Arber (1925) commenting on the nodal structure of grass culms stated that 'the task of following the bundles in the monocotyledonous axis owing to the crowding of the leaf traces and their varying curvatures . . . is an exacting and difficult task which botanists seem to have shirked accordingly; indeed, most of our information on the subject is many years old. It is much to be desired that some present-day worker would apply the skill and patience necessary for obtaining fresh data with the aid of modern technique' (Chap. III, p. 37). In 1934 she stated again that 'in the internode of grass haulms the course of bundles is vertical, but at the level of the nodes horizontal strands are met with which connect the vertical strands and anastomoses into an elaborate nodal plexus' (p. 259, Figs. 125, B₁, B₂).

The position, it appears, remained the same in 1947 when Eames and MacDaniels wrote that 'in monocotyledons the many bundles form extremely complex nodal structures . . . There is little clear information as to the basic plan in these nodes' (p. 152). It seems strange that they (Arber, 1925, 1934; Eames and MacDaniels, 1947) did not make any reference to the works of Percival (1921) and Sharman (1942).

In 1921 Percival studied the vascular system in the internode, node and leaf base in the shoot of wheat plant which is hollow like that of the rice internode. Sharman (1942) studied the same in the maize plant, the internode of which is solid. (For detailed information see authors' original papers, or Esau, 1953, pp. 399-402.)

* Now in the University of Manchester, England.

Esau (1953), however, makes a general comment that 'the complex arrangement of the vascular bundles (in grass haulm) is related to the variable orientations of the different traces of the same leaf' (p. 400).

Stover (1951) distinguished three types of grass stem on the basis of their internodal structures, namely, (1) in the *Fastuceae*, *Hordeae* and in some species of *Agrostideae* the stem bundles are arranged in a single cylinder, (2) in the *Agrostideae* and *Paniceae* they are arranged in two or three cylinders, and (3) in the *Andropogoneae* and *Maydeae*, the stems of which are solid, the bundles are irregularly arranged and no cylindrical arrangement is noticed (p. 178). Esau (1953) on the other hand recognized two basic plans in the distribution of vascular bundles in the internodes of grass stem: (1) in hollow internodes the bundles are arranged in two rings or circles, the smaller bundles nearer the periphery, and the larger ones somewhat deeper in the stem, as in *Triticum*, *Oryza*, etc., and (2) in the solid internodes the bundles are scattered throughout the ground tissue, as in *Zea*, *Sorghum*, *Bambusa*, etc. (p. 399).

In rice internodes the fibrovascular bundles are arranged in two circles as in wheat plant; sclerified hypodermis forms a continuous stereome cylinder with the bundles of the outer ring more or less embedded in it. The nodal structure is very complex.

So far as we are aware no detailed studies of the vascular system in the shoot of *Oryza* have been made. As our studies of the nodal and internodal structures of this plant show considerable variation from those of Bugnon (1920a) and Percival (1921) in many details the present communication is considered desirable.

MATERIALS AND METHODS

Materials for the present studies were grown in the botanical garden of Dacca University. To study the vascular system of the mature plant a few seedlings were transplanted in pots. Plasticine cups were fixed to the base of the mature internodes above ground level below a node and a weak solution of India ink was poured in the cups. By means of a sharp scalpel a transverse puncture was made in the stem under the ink solution in some cases. The black solution was sucked in slowly through the vascular bundles, and then the course of some particular bundles could be easily traced in the stem through the internodes and nodes by serial transverse sections through them. The nodal regions with leaf-sheaths and portions of the lower and upper internodes of the seedlings and adult plants were fixed and preserved in FAA solution. At the time of use the preserved materials were dehydrated and embedded in paraffin according to schedule. Some of the sections were microtomed at 10μ – 15μ , and stained in safranin and fast green combination, but most of the sections were cut free hand or with a hand microtome. Hand sections were stained with a light solution of safranin and serially mounted in 20% glycerine solution and sealed with a paraffin-rubber preparation.

Text-figures (Figs. 1–15) have been drawn under a camera lucida. Photomicrographs (Figs. 16–23) have been taken only of the salient stages wherever thought necessary.

OBSERVATIONS

As we believe in the acropetal differentiation of the provascular meristem in the shoot of higher plants the vascular system in the shoot of *Oryza* has been studied in serial transverse sections from the top of an internode upwards through the node to the base of the next upper internode.

In the hollow internode the bundles show considerable regularity in their distribution and form two distinct circles around the stem. The outer ring consists of small vascular bundles which are more or less embedded in the hypodermal stereome tissue. All these bundles are not similar in size but are alternately large

and small. Their number varies from 18 to 22 in this ring. Beneath the outer zone of bundles occurs a median zone (inner circle) which contains the same number of bundles which are much bigger in sizes. In this ring also the bundles exhibit two types: the ovals and the ellipticals (roughly on their shape). The ovals are situated just opposite the big bundles of the outer ring, and the ellipticals to the smaller ones (Figs. 1, 2). The ellipticals are slightly bigger than the ovals. Thus the bigger of the inner ring are situated opposite to the smaller of the outer and vice versa. In this way *four types* of vascular bundles (Figs. 2 and 3) can be distinguished in the internode by their size and position. Each leaf has numerous trace bundles: large, small and smallest arranged in the order—large, smallest, small, smallest, large and so on in parallel series (Figs. 22, 23).

In order to follow in detail the course of internodal bundles through the node to the base of the leaf and to that of the next upper internode three consecutive bundles of the outer ring (numbered 1, 2, 3) and three of the inner ring (numbered 4, 5, 6) have been selected (Fig. 2).

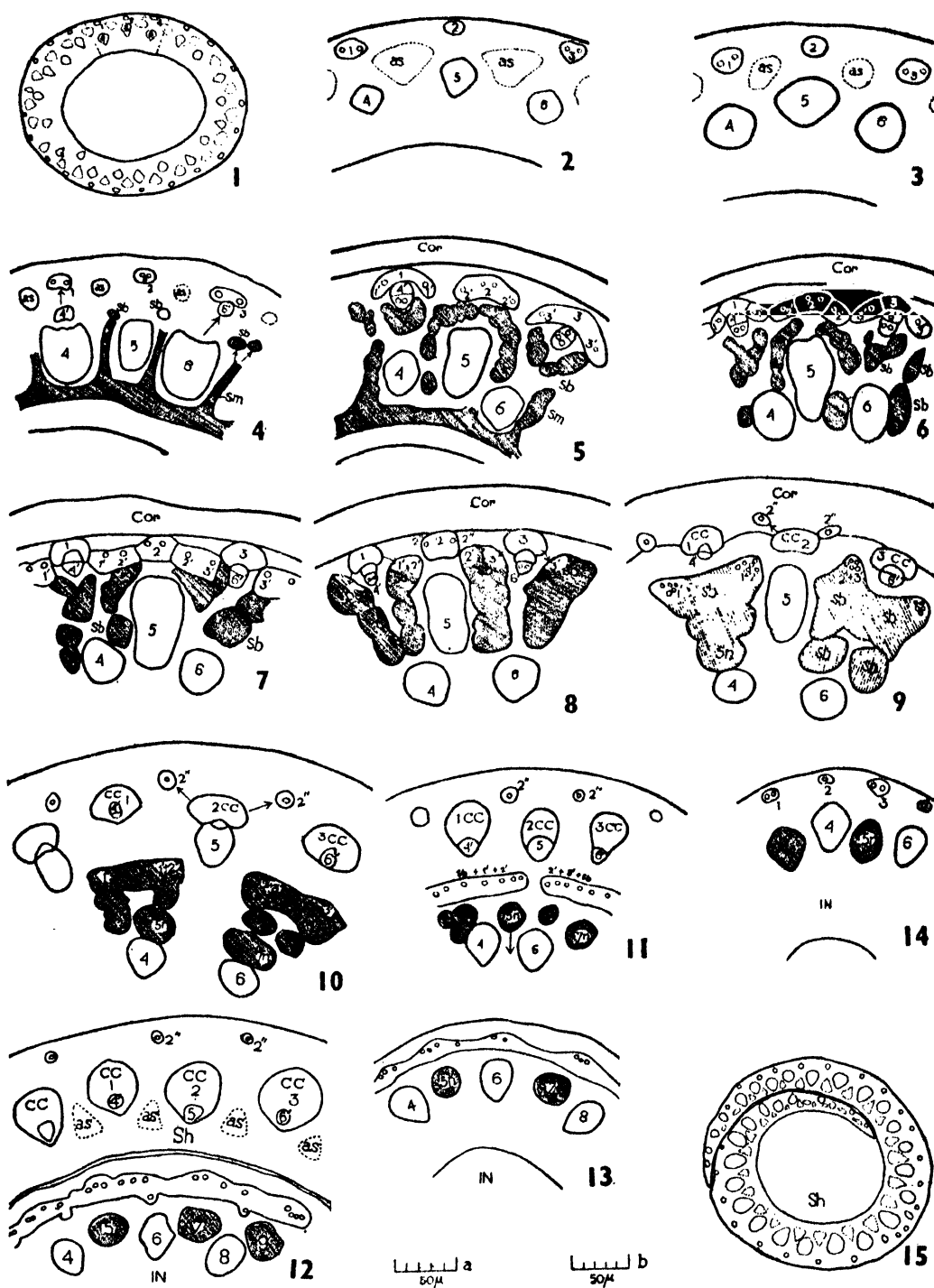
As we proceed from the internode towards the base of the node the vascular bundles increase in size and the air cavities gradually become smaller and smaller and ultimately disappear in the nodal region (Figs. 3–5). At first the inner ovals (4, 6) and the outer bigger bundles (1, 3), placed opposite to each other, enlarge, but the inner elliptical (5) and the outer smaller (2) retain somewhat their normal size with slight changes in their shape (Fig. 3). Each of the outer bigger bundles (1, 3) becomes laterally extended with the two xylem elements shifted towards their ends. The inner ovals (4, 6) give out a branch each (4', 6') towards the periphery which later on join the central region of bundles 1 and 3 (Figs. 4–6 and 17–19). Transverse provascular meristem develops in enormous quantity and forms a network completely surrounding the vascular bundles of the inner ring. By this time four vessel elements differentiate in the outer smaller bundle 2 which has also extended laterally (Figs. 5 and 6).

The ovals (4, 6) gradually regain their normal size and move towards the centre of the nodal plate, when the elliptical (5) enlarges (Figs. 5–8, 17 and 19). All the bundles of the outer ring become semi-lunar enlarging laterally (Figs. 6 and 17). Bundles 1 and 3 by this time join with the branches of the ovals (Figs. 5, 6 and 18). In serial transverse sections through the node many large and small subsidiary bundles are seen to differentiate by the breaking up of the secondary provascular meristem in the node (Figs. 4–11 and 18–22). An outer zone of cortical tissue free of vascular bundles is now discernible which represents the continuation of the sheathing leaf-base in the node (Figs. 5–9 and 17–22).

The elliptical (5) continues to enlarge and moves towards the periphery to join the outer smaller (2) at its centre. All the bundles of the outer ring, big and small, can now be distinguished into three parts—one central and *two* marginal (1', 1, 1'; 2', 2, 2'; 3', 3, 3'; Figs. 5, 6, 18 and 19). The marginal parts have one xylem element each, but the central part of the bigger bundle has none whereas that of the smaller one has two elements each (Figs. 7, 8 and 18). The central parts of the bigger bundles (1, 3) later form the collenchymatous caps of the branches from the inner ovals (4', 6'; Figs. 9 and 10).

We have already mentioned that the ovals move towards the centre and the enlarged elliptical towards the periphery of the node. The contiguous marginal parts of the bundles of the outer ring (1, 2, 3) unite with one another and also with strands of secondary origin (Figs. 8–10 and 20). The central part of the outer smaller bundle (2) having two vessel elements now gives out two branches (2'', 2'') containing one vessel element each towards the base of the leaf-sheath, leaving the central region without any xylem element. This part like the central parts of 1 and 3, forms the collenchymatous cap of the elliptical 5 (Figs. 9–11 and 20–22).

The three composite bundles (4'+1, 6'+3, 5+2) formed by the union of the branches of 4 and 6 with the central parts of 1 and 3, and of the elliptical 5 with



Magnifications of Figs. 2-14, and of 1 and 15 are indicated respectively by the scales *a* and *b* at the bottom of the figures.

(For Explanation of Text-figures, see p. 240.)

the central part of 2, now move towards the base of leaf-sheath. The branches, 2" and 2" of 2, also move independently to the sheathing leaf-base. The composite bundles forming the large bundles of the leaf-sheath have enormous quantity of collenchyma around them to give flexibility to the sheathing base (Fig. 23).

The lateral parts of all the bundles of the outer ring now separate from the centre and move inwards (1', 1'; 2', 2'; 3', 3'), unite and in combination with the subsidiary vascular tissues form a vascular cylinder broken only in the region through which the ellipticals pass out to the base of the leaf-sheath (Figs. 11 and 22). The gap in the inner ring caused by the departure of 5 is later filled up by a bundle formed anew from the nodal meristem and moved into the gap to complete the ring (Figs. 10-12). The ovals gradually change their shape to become the ellipticals in the next upper internode (Figs. 12-14).

The sheathing base of the leaf now separates from the node (Figs. 12 and 23). The discontinuous outer vascular cylinder is then repaired with secondary vascular tissues and a complete cylinder is formed (Figs. 12 and 13). It then breaks up into units which organize as large and small bundles of the outer ring in the next upper internode (Fig. 14). This happens at the base of the upper internode. Thus the peripheral bundles of an upper internode are made up of the lateral vascular parts of the outer bundles of the immediately lower internode with some amount of the subsidiary vascular tissues of the node. Half the number of bundles of the inner ring of an internode come from the lower internode (ovals which change their shape

EXPLANATION OF TEXT-FIGURES ON P. 239 (for description see text)

(All the figures have been drawn and photomicrographed from serial transverse sections of the axis from the top of an internode to the base of the upper internode through the intervening node)

Fig. 1 shows the arrangement and disposition of the vascular bundles of the internode. Fig. 2 is a portion of Fig. 1 showing distribution of bundles 1, 2 and 3 of the outer ring, and 4, 5 and 6 of the inner ring, and of air spaces. Fig. 3 represents the structure of the internode just below the node showing enlargement of the bundles, and reduction in the size of air spaces before they enter the nodal region. Fig. 4 is a t.s. of the node showing origin and the spread of the provascular meristem and differentiation of a few subsidiary strands from it; it also shows branchings of 4 and 6 (4', 6'). Figs. 5 and 6 transverse sections of the node higher up showing demarcation of the cortical region, lateral spread of the outer internodal bundles, disintegration or breaking up of the secondary vascular tissues into several parts, and the union of 4' and 6' with the central parts of 1 and 3; bundle 5 has enlarged further and has moved towards 2. Fig. 7 is a t.s. of the node showing coalescence of the contiguous lateral parts of 1, 2 and 3, and with subsidiary vascular tissues. Figs. 8 and 9 show the separation of the coalesced parts from the central parts of 1, 2 and 3 and their movement inwards; outward branches 2" and 2" of bundle 2 organize and separate from it leaving the central part without any vascular elements; 5 has moved out further, and 4 and 6 have moved towards the centre of the node. In Fig. 9 new bundle 5n is being organized from the secondary vascular meristem. Figs. 10 and 11 show the full organization of the three types of leaf-trace bundles, and their movement outwards into the cortical region; a cylinder of vascular tissue has formed with the branches of 1, 2 and 3 and some amount of subsidiary vascular tissues broken at the place through which 5 has passed out; 5n has moved to occupy its position between 4 and 6. Fig. 12 shows the separation of the leaf-sheath with its trace bundles and air spaces from the node; the broken cylinder of vascular tissues has been repaired, and the new bundles have taken their position in the inner ring. Figs. 13 and 14 show that the complete ring has now broken into its component parts, the small and large bundles of the outer ring of the upper internode. Fig. 15 is the t.s. of the entire sheath shown in part in Fig. 12.

LEGEND

as—air space; cc—collenchymatous cap; cor—cortical region; in—upper internode; sb—subsidiary bundle; sh—leaf-sheath; sm—secondary provascular meristem; 5n—new bundle of the inner ring (nodal origin); cross hatchings indicate origin from secondary provascular meristem of the node.

to become ellipticals), and the other half are formed from the meristem of the node between the two successive internodes. Each new bundle thus originated passes through two consecutive internodes and then moves out to the base of the leaf-sheath in the third node from its node of origin.

As the structure of the nodes and the vascular organization in the shoot of *Oryza* are rather complex and confused, a summary of the observations recorded above is given below:

- A. *Internodal bundles (18-22 in each ring)*: They are arranged in two concentric rings, and in each ring there are two types of bundles, large and small, alternating with each other. We have followed the course and behaviour of six bundles, three of the outer (numbered 1, 2, 3) and three of the inner (numbered 4, 5, 6) circles through the node from the top of the lower internode to the base of the next upper internode, and to the sheathing base of the leaf. Redundant details have been omitted to follow their courses clear in the shoot.
1. *The outer large bundles (1 and 3)*: On entering the node from below each of them organizes into three distinct parts, the central without any vascular tissue (1', 1, 1'; 3', 3, 3'). The lateral parts with the vascular elements separate from their central and join the contiguous similar parts of 2 (2', 2'), and later together with the subsidiary vascular tissues form the bundles of the outer ring of the next upper internode. The central parts of 1 and 3, which are non-vascular, form the collenchymatous caps of the branches of 4 and 6 (4', 6') and go to the leaf as its *small* bundles.
 2. *The outer small bundle (2)*: It gives out *four* lateral branches, two towards the centre and two towards the periphery of the stem. The two inward branches (parts 2', 2') go to form the vascular bundles of the outer ring of the next upper internode in conjunction with the laterals of the outer large (1', 3') and subsidiary bundles. The two outward branches (2'', 2'') go to form the intermediate *smallest* bundles of the leaf-sheath. The central portion of 2, now devoid of any vascular tissue, forms the collenchymatous cap of the elliptical 5 of the inner circle, and the two together pass out to the base of the leaf-sheath as its *large* bundle.
 3. *The inner ovals (4, 6)*: They give out one small branch each (4', 6') towards the periphery to join the non-vascular central parts of 1 and 3 respectively and pass out as the *small* bundles of the leaf-sheath. The rest of the ovals go to the next upper internode as ellipticals of its inner ring.
 4. *The inner elliptical (5)*: It moves towards bundle 2 which it ultimately joins, and along with the non-vascular central part of the latter as its collenchymatous cap forms the large bundle of the leaf-sheath.
- B. *Leaf-sheath bundles*: They comprise *three* types, namely, the smallest, the small and the large, arranged in the order—large, smallest, small, smallest, large, and so on in parallel series.
1. *The smallest (intermediate) bundles*: They are branches (2'', 2'') of the outer internodal bundle 2. The number of such intermediate bundles are twice the number of bundles branched.
 2. *The small bundles*: These are composite bundles, each being composed of the central non-vascular parts of 1 and 3, and a branch from the corresponding inner ovals, 4 and 6.
 3. *The large bundle*: It is also a composite bundle being composed of the non-vascular part of 2 and the entire elliptical 5 of the lower internode.

C. *The nodal plate*: The origin, nature and composition of the vascular system of the nodal region have been worked out as follows:

1. Secondary provascular meristem takes its origin in the nodal region in enormous quantity, spreads in all directions and surrounds the vascular bundles of the inner ring of the internode below; from this nodal meristem transverse, vertical and radial secondary vascular tissues differentiate,
2. branchings of the bundles of the outer ring and of the ovals of the inner ring of lower internode take place,
3. all the bundles grow in size,
4. the ovals after sending out a branch each change their shape to become ellipticals of the next upper internode,
5. new ovals of the upper internode are formed from the secondary meristem, and they move into the gaps caused in the inner circle by the wholesale departure of the ellipticals for the base of the leaf-sheath,
6. many additional bundles also differentiate from the secondary meristem, but they disappear at the base of the upper internode,
7. interconnections and anastomoses take place among the internodal vascular bundles of the outer and inner rings of the internode by means of secondary vascular tissues and their branchings,
8. vascular parts of the outer bundles coalesce and with subsidiary vascular tissues form a complete cylinder before they break up into large and small bundles of the outer ring of the upper internode,
9. the leaf-trace bundles depart for the base of the leaf-sheath, and
10. all air cavities and the hollowness of the pith disappear.

D. *Upper internodal bundles*: The bundles of the outer ring come from the branches (lateral vascular parts) of both the small and large bundles (1, 2, 3) of the outer ring of the internode below. But before they separate into discrete bundles in the upper internode they form along with the subsidiary tissues a complete cylinder in the upper region of the node. The ellipticals of the inner ring are the ovals of the internode below and the ovals are formed anew from the secondary provascular meristem of the node.

DISCUSSION AND CONCLUSION

The nodal structure of the grass stem has been described by previous workers as complex and confused. Esau (1953) thinks that such complexities are due 'to the variable orientation of the different *traces of the same leaf*'. She thus supports the view held by Bugnon (1920a) in the case of rice seedlings. Eames and MacDaniels (1947) write 'there is little clear information as to the basic plan in these nodes'. Esau (1953), however, distinguished two basic plans of distribution of the vascular bundles in the two types of internodes, hollow and solid, though Stover (1951) classified three types of grass stem on the basis of their internodal structure.

In 1921 Percival investigated the nodal and internodal structures of the wheat plant, with hollow internode and solid node. Sharman (1942) worked out the same structures in the solid node and internode of the maize plant. So it will be seen that the vascular systems of the grass shoot with hollow internode and solid node have not been studied in detail after 1921. The present studies of the same in the shoots of the rice plant may be regarded as a new contribution.

In the present studies the origin and course of internodal bundles have been followed from the upper region of the lower internode to the base of the leaf-sheath

diverging from the node and to that of the next upper internode through the intervening node. It would thus be seen that the course of the vascular system has been followed aeropetally in the shoot of *Oryza* instead of basipetally as has been done in the case of *Triticum* by Percival.

The internodal bundles of *Oryza* stem, as in that of *Triticum*, are arranged in two circles: an outer peripheral and an inner internal. By their size and shape the bundles of the outer ring are distinguished into large and small, and those of the inner ring roughly into ovals and ellipticals. The inner are the largest bundles of the stem. In their respective rings they alternate with one another, and in their disposition the large and small bundles of the outer ring are placed opposite respectively to the ovals and the ellipticals of the inner ring. In the nodes the ovals and all the bundles of the outer ring branch. Each of the former sends out a small branch to join the central parts of the outer large bundles and the two together form the trace bundles (small) of the leaf. The ellipticals do not branch, but move out to join the central parts of the outer small bundles and form the largest leaf-trace bundles. Each of the small bundles of the outer ring sends out two branches to form the smallest trace bundles of the leaf. The central parts of the outer bundles do not contain any vascular tissue and they form the collenchymatous caps of the small and largest bundles of the leaf-sheath. Therefore the leaf-trace bundles are not only large, small and smallest according to their size, but they differ also from one another in their origin and composition.

Each large bundle of the outer ring (internodal) extends laterally and inwardly to assume the form of an arc. The vascular elements move to the ends leaving the central part without any vascular tissue. At this stage each bundle can be divided into three parts: the central without any vascular tissue and two lateral parts with vascular elements. This also happens in the case of the small bundles, but their central portions still retain two vascular elements each which later go with the branches which they send out to form the smallest bundles of the leaf. Their central parts, like those of the large bundles, now become non-vascular. It is thus to be noted that the central parts of the outer bundles become non-vascular before they are converted into collenchymatous caps. It is also clear that the large and small bundles behave differently in this respect.

The lateral vascular parts then separate from the non-vascular central ones, and the contiguous parts of the neighbouring bundles, one from the large and the other from the small, unite first with each other and then with the secondary vascular tissues to form a broken cylinder. Just above the level of separation of the leaf the broken cylinder becomes continuous which then breaks up into alternate large and small outer bundles of the upper internode. These bundles are therefore lateral vascular parts of the peripheral bundles of the lower internode. They remain confined to the stem and send out only the non-vascular central part to the leaf.

At each node the ellipticals pass out to the leaf causing gaps in the inner ring. Their places are taken by bundles formed anew from the provascular meristem of the node. The ovals while passing upwards through the node give out a branch to the leaf and then change their shape to become the ellipticals, and the new bundles which take the position of the departed ellipticals enter the upper internode as its ovals.

Thus the bundles of the inner ring, which are the largest in the stem, are all leaf-traces, take their origin in the provascular meristem of the node, pass upwards through two consecutive internodes, and move out to their leaves at the third node from the node of their origin.

The origin of the provascular meristem in the node is secondary, and is a peculiar feature of the grass stem. It differentiates into a transverse vascular system which along with the leaf-trace bundles and their branches form the complicated nodal plexus. It spreads transversely in all directions but keeps inside

the ring of outer bundles, surrounds the inner bundles half of which take their origin in this meristem to replace those which leave for the leaf diverging from the node. The vascular bundles, differentiated from this meristem anastomose, form a network and establish interconnections between all the bundles of the internodes and their branches, and finally disappear in the upper region of the node.

The provascular meristem is thus independent in origin and is confined entirely to the node. Our studies support Mohl (Bugnon, 1920*a*) but do not support Bugnon (Bugnon (*Oryza*), 1920*a*, 1920*b*, 1924; Sharman (*Maize*), 1942), though it is true that outer internodal bundles (small and large bundles in *Oryza*) divide in the nodes into vascular and non-vascular parts, and the former unite with the secondary vascular tissues to form a continuous cylinder in the upper region of the node. It is to be noted that the outer internodal bundles are really cauline as they contribute only their central non-vascular parts (residual) to form the collenchymatous caps of the leaf-trace bundles and half of them (small) send out branches to form the intermediate bundles of the leaf-sheath. The continuity of the peripheral bundles from internode to internode is maintained by their vascular lateral parts to which some secondary tissues may be added.

Independence of the transverse system from the buds is supported (Bugnon, 1924; Esau, 1953, pp. 400-402, Figs. 15 and 23, A-F). The complicated structure of the node is not due to the variable orientation of the different traces of the same leaf (Bugnon, 1920*a*; Esau, 1953). This may be true for *Triticum*, but the same cannot be said of *Oryza*. The nodal plexus in the rice plant, as we have already seen, is made up of a system of transverse vascular tissues differentiated from a provascular meristem of secondary origin, combined with the leaf-trace bundles of two successive leaves, and their branches *plus* the cauline bundles of the peripheral ring, and their branches. All these bundles anastomose, form a network, and establish interconnections with one another.

Anatomically the node which is vertically thick (leaf cushion) can be distinguished into three regions: lower, middle and upper. In the basal region the provascular meristem differentiates; in the middle region the nodal plexus is formed including the origin of new leaf-trace bundles, and branchings of the internodal bundles, and in the upper region the bundles of the outer internodal bundles are constituted. Therefore Sharman's (1942) finding that the node of maize stem is made up of the base of the upper internode and the upper half of the disc of insertion of the leaf primordium seems substantially correct also in the node of *Oryza*.

The swollen nature of the node is easily explained because the disc of insertion (leaf cushion) of the leaf primordium (cf. maize stem, Sharman, 1942) encloses the axis, and gives the maximum thickness to the node.

SUMMARY

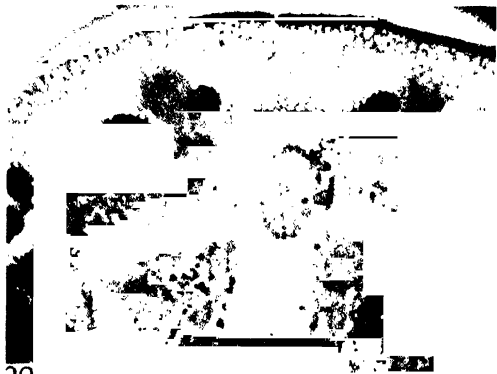
The origin, nature and the course of the vascular system in the shoot of rice plant have been studied somewhat in detail in serial transverse sections from the top of a lower internode to the base of the next upper internode through the intervening node and also to the base of the leaf diverging from the latter.

In the internode the bundles are arranged in two concentric rings in which they are alternately large and small in the outer, and roughly oval and elliptical in the inner rings respectively. The ovals and ellipticals are the largest in the stem, and are placed opposite respectively to the large and small bundles of the outer ring.

In the node each oval sends out a small branch which with the non-vascular central part of the outer large bundle as its collenchymatous cap goes to the leaf as its small bundle. The oval then moves up to become the elliptical of the upper internode. The elliptical on the other hand passes entirely to the leaf with the non-vascular part of the outer small bundle, as the largest leaf-trace bundle. Its place in the node is taken by a new bundle originated from the secondary meristem of the node. This new bundle then passes up to the upper internode as the oval of its inner ring. All the inner bundles, ovals and ellipticals, are therefore leaf-traces, and one half of them by turn take their origin in the nodal meristem. Each of them passes through two successive internodes alternately as the oval and the elliptical before it passes out in its leaf.



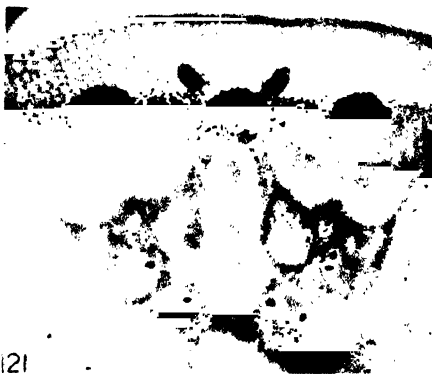
16



20



17



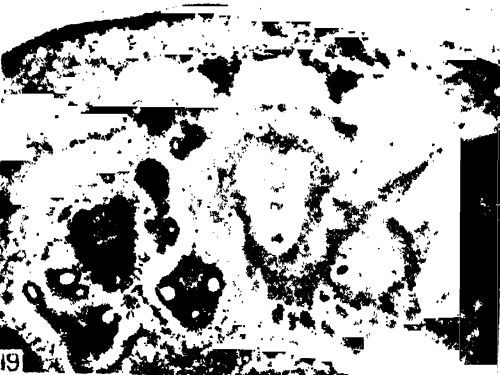
21



18



22



19



23

The bundles of the outer ring behave differently. The large bundles extend laterally in which the vascular elements move leaving the central parts without any vascular tissue. The small bundles in addition to dividing into three parts like the large bundles also send out two branches, each with one vascular element in each branch, to the leaf as the latter's smallest bundle. Their central parts now become non-vascular. Thus the three kinds of leaf-trace bundles, largest, small and smallest, not only differ in their sizes but also in their origin and nature.

The lateral vascular parts of each bundle, small and large of the peripheral ring, separate from the central non-vascular parts and move inwards. The contiguous parts of the neighbouring small and large bundles unite and then coalesce with the secondary tissues differentiated from the nodal meristem to form a complete cylinder in the upper region of the node before the latter breaks up into small and large bundles of the outer ring of the upper internode. These bundles or their vascular parts continue from internode to internode, and only the non-vascular central parts are sent to the leaf as the collechymatous caps of the leaf-trace bundles of the large and small, and two branches from each of the small bundles as the intermediate bundles of the leaf-trace.

The nodal structure, which has been described as complex and confused, is the result of the origin of the transverse provascular meristem in the node. This meristem spreads in all directions and surrounds the inner leaf-trace bundles and differentiates into a transversely oriented vascular system which forms a network and establishes intercommunications between all the bundles of the node, and their branches. The secondary bundles originate and disappear with the exception of half the number of leaf-trace bundles, which it gives origin to, within the node.

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EXPLANATION OF PLATE XXIII

Fig. 16 shows the origin and spread of the provascular meristem at the base of the node. Figs. 17, 18 and 19 are t.s. of the node at different levels showing distribution of the subsidiary bundles, movements of the ovals and their branches, of the elliptical, and of the lateral spreads of the outer bundles. Figs. 20, 21 and 22 show the origin and separation of the outward branches of 2; formation of broken cylinder of vascular tissues (cf. Fig. 11) and the origin of the new bundles of the inner ring to replace those left for the base of the leaf. Fig. 23 shows the separation of the leaf-sheath with its bundles from the node; air spaces have appeared; the upper internodal bundles are still being reconstituted at the base of the upper internode.

CHROMOSOME STUDIES IN SOME INDIAN BARLEY. I

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CONTENTS

	Page
Introduction	246
Materials and Methods	247
Observations	247
Meiosis	250
Discussion	251
Acknowledgements	253
Summary	253
References	253

INTRODUCTION

The genus *Hordeum* has attracted the attention of cytologists from time to time, and a good deal of work has been carried out regarding the chromosome counts and the meiotic behaviour in different species of the genus. Different authors, viz. Kihara (1924), Kagawa (1929), Ghimpu (1929) and Tsuchiya (1952), reported the diploid chromosome number as fourteen in case of *H. vulgare* Linn. Lewitsky (1931) observed two pairs of chromosomes with satellites in the body cell of all cultivated diploid species of *Hordeum*. Chin (1941), too, corroborated his observation after a study of the morphology of the chromosomes in all cultivated diploid forms. In case of wild diploids and tetraploids, he recorded the occurrence of one pair of satellited chromosomes in the former and two pairs in the latter. Attempts towards the induction of higher polyploids of barley have also been met with marked success (Chen *et al.*, 1945). The meiotic behaviour of chromosomes of all such raised types has also been thoroughly studied. Critical study to some extent in the cereals has been carried out by Oinuma (1952).

The common Indian barley, *Hordeum vulgare* Linn., is cultivated throughout the drier parts of India and different agricultural strains have been raised in the Indian Agricultural Research Institute, New Delhi. Though much of genetical work has been done on the species, no attempt has yet been made to make a critical investigation into the cytogenetics of the different strains of barley as well as *H. murinum* L. and *H. distichon* L., the two allied species of *H. vulgare*.

The fact that the genus *Hordeum* represents plants of much economic importance and that its species and varieties, having chromosomes quite small in number and large in size, provide good material for a critical study of their morphology, an aspect of study quite unattempted till now, it was thought desirable to carry out a critical investigation into the cytogenetics of its different species and strains with the aid of the recently improved technique. With this end in view, the present investigation was undertaken. The present paper deals with the karyotype analysis and the meiotic behaviour of different strains of barley, obtained from the Indian Agricultural Research Institute, New Delhi.

MATERIALS AND METHODS

The following strains of barley have been used in the present investigation:

(1) IP: 1, (2) IP: 9, (3) IP: 13, (4) IP: 14, (5) IP: 20, and (6) IP: 24.*

For the study of somatic chromosomes, root tips were fixed from seeds germinated in sawdust in the laboratory. After a strenuous trial in different fixing fluids, it became apparent that the metaphase chromosomes of these strains, though properly fixed, presented much difficulty in the interpretation of their morphology due to their considerable length as well as due to the presence of much foreshortening of their arms. For the purpose of straightening the chromosome arms, simultaneously with the exaggeration of the constrictions present, it was thought desirable to treat the root tips, before fixing, in cold temperature in dilute aqueous solution of colchicine. The application of this colchicine technique for the purpose of chromosome straightening has already been met with marked success in case of *Vicia faba* L. by Bhaduri (1939). After a series of trials, best results were, however, obtained by fixing the root tips between 12 noon and 1 p.m. in a solution of 1% aq. platinic chloride and 10% formalin in the proportion of 1:4, after a pretreatment in 0.5% colchicine solution for one hour and then subsequently washing in water for the same period. It was observed that a treatment in slightly lower dilution of colchicine for a longer period and in slightly higher dilution for a shorter period yielded more or less the same results. In some of the cells, however, tetraploid number of chromosomes were observed, formed as a result of direct effect of colchicine. For the study of meiosis, flower buds were collected from the plants grown in the Calcutta University Botanic Gardens, and fixed between 10 a.m. and 11 a.m. in Nawaschin's fixative after a pretreatment in Carnoy's fluid.

Sections were cut at a thickness of 18μ both in case of flower buds and root tips and stained in the usual procedure of Newton's crystal violet-iodine technique. In case of root tips, however, hydrolysis in N. HCl for fifteen minutes at 60°C . and then premordanting in 1% chromic acid for overnight became necessary to bring out best results. For the study of nucleoli, Feulgen-Light green technique of Semmens and Bhaduri (1941) was followed.

The figures were drawn at a table magnification of 3,600 times approximately using a compensating eyepiece $\times 18$ and a 1.3 N.A. apochromatic objective.

OBSERVATIONS

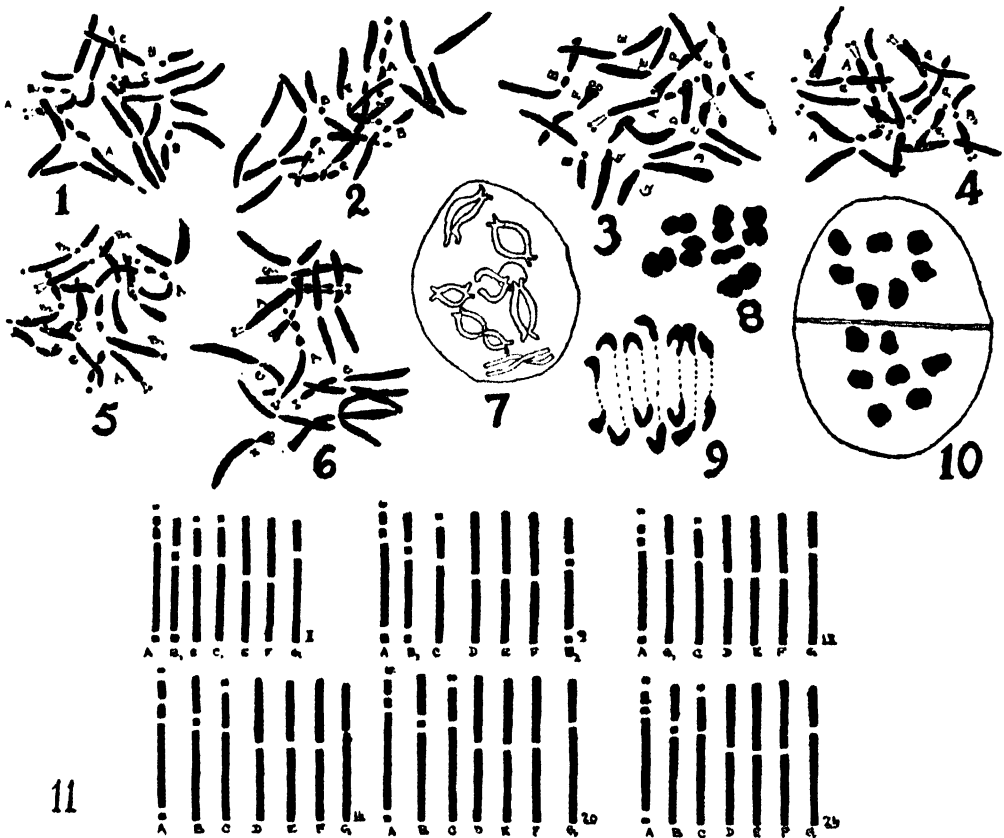
The chromosome numbers of all the strains studied were found to be fourteen, thus corroborating the previous observations (Lewitsky, 1931; Chin, 1941, etc.). The karyotype analysis of all these strains revealed much difference in chromosome morphology in at least some of them, though the presence of supernumerary constrictions in the chromosome is a constant feature of all the strains studied so far. The karyotype of the strains fourteen, twenty and twenty-four were found to be identical with each other, whereas the other three strains, viz. one, nine and thirteen, showed differences in chromosome morphology among themselves as well as from the common karyotype of the strains fourteen, twenty and twenty-four (Fig. 11). The complements in all of them are characterized by having fairly long chromosomes, the size difference between themselves being not very marked. The lengths of the chromosomes were found to vary from 10.5μ to 8.3μ .

* IP = Old Pusa or Imperial Pusa.

I. *Karyotype of the strains IP: 14, IP: 20 and IP: 24*

(Figs. 1, 2, 3 and 11)

The seven pairs of chromosomes could be classified into five distinct types which are as follows (lengths of the chromosomes vary from 10.5μ to 9.8μ):



FIGS. 1 TO 10—Somatic metaphase plates of the strains IP—14, 20, 24, 1, 9, 13 and meiotic stages of IP—24 respectively.

FIG. 11—Karyotypes of the strains IP—1, 9, 13, 14, 20 and 24 respectively.

Chromosomes with secondary constrictions :

- (1) A pair of long chromosomes with nearly submedian primary constrictions and a satellite at the long arm. In the short arm, however, one median secondary constriction and a satellite at the end are also present, thus making the total number of constrictions, excluding the primary one, present in the pair as six (AA).
- (2) A pair of medium sized chromosomes, with nearly submedian primary constrictions and a secondary constriction very near the primary one, located on the shorter arm (BB).
- (3) A pair of medium sized chromosomes with nearly submedian primary constrictions and a satellite at the end of the short arm (CC).

The total number of constrictions, excluding the primary ones, located in these three pairs is, therefore, ten.

Chromosomes not with secondary constrictions :

- (4) Three pairs of medium sized chromosomes with median primary constrictions (DD, EE, FF).
- (5) One pair of medium sized chromosomes with submedian primary constrictions (GG).

II. *Karyotype of the strain IP : 1 (Figs. 4 and 11)*

The karyotype of this strain may be classified into the following distinct types (lengths of the chromosomes vary from 9.4μ to 8.3μ) :

Chromosomes with secondary constrictions :

- (1) A pair of long chromosomes with nearly submedian primary constrictions and a satellite at the end of the long arm. In the short arm, a median secondary constriction and a satellite at the end are also present, thus making the total number of constrictions, excluding the primary one, present in the pair as six (AA).
- (2) A pair of medium sized chromosomes, each with a submedian primary constriction and a secondary constriction very near the primary one towards the shorter arm. A satellite is also present at the end of the long arm, thus making the total number of constrictions, excluding the primary one, present in the pair as four (B_1B_1).
- (3) Two pairs of medium sized chromosomes, each with a submedian primary constriction and a satellite at the end of the short arm (CC, C_1C_1).

The total number of constrictions excluding the primary ones present in these four pairs is, therefore, fourteen.

Chromosomes not with secondary constrictions :

- (4) Two pairs of medium sized chromosomes with nearly median primary constrictions (EE, FF).
- (5) A pair of medium sized chromosomes with nearly submedian primary constrictions (GG).

III. *Karyotype of the strain IP: 9 (Figs. 5 and 11)*

The karyotype of this strain may be classified into the following distinct types (lengths of the chromosomes vary from 9.8μ to 8.6μ) :

Chromosomes with secondary constrictions :

- (1) A pair of long chromosomes, each with a submedian primary constriction and a satellite at the end of the long arm. The short arm is provided with a median secondary constriction and a satellite at the end, thus making the total number of constrictions, excluding the primary ones, present in the pair as six (AA).
- (2) Two pairs of medium sized chromosomes, each with nearly submedian primary constriction, a secondary constriction very near the primary one towards the short arm, and a satellite at the end of the long arm. The total number of constrictions, excluding the primary ones, present in these two pairs is, therefore, eight (B_1B_1 , B_2B_2).
- (3) A pair of medium sized chromosomes, each with nearly submedian primary constriction and a satellite at the end of the short arm (CC).

The total number of constrictions, excluding the primary ones, present in these four pairs is, therefore, sixteen.

Chromosomes not with secondary constrictions :

- (4) Three pairs of medium sized chromosomes with nearly median primary constrictions (DD, EE, FF).

IV. *Karyotype of the strain IP : 13 (Figs. 6 and 11)*

The karyotype of this strain can be classified into the following distinct types (lengths of the chromosomes vary from 9.4μ to 8.9μ):

Chromosomes with secondary constrictions :

- (1) A pair of long chromosomes, each with submedian primary constriction and a satellite at the end of the long arm. The short arm also possesses a median secondary constriction, and a satellite at the end, thus making the total number of constrictions, excluding the primary ones, present in the pair as six (AA).
- (2) A pair of medium sized chromosomes, each with submedian primary constriction and a satellite at the end of the short arm (CC).

The total number of constrictions, excluding the primary ones, located in these two pairs is, therefore, eight.

Chromosomes not with secondary constrictions :

- (3) Three pairs of medium sized chromosomes with nearly median primary constrictions (DD, EE, FF).
- (4) Two pairs of medium sized chromosomes with nearly submedian primary constrictions (GG, G_1G_1).

All these strains showed the presence of a high number of nucleoli, and as many as ten in case of strain IP: 9, in the telophase stage of root tip cells, showing that all the constrictions, excluding the primary ones, are *probably* nucleolar in nature. This aspect of study involving chromosome-nucleolus relationship has not, however, been carried out yet. In any case, the nature of these constrictions, whether nucleolar or non-nucleolar, is yet to be ascertained.

MEIOSIS

Meiotic behaviour of chromosomes so far studied proved to be more or less similar in nature in all the strains examined, being fairly regular in nature. During diplotene stages, both interstitial and terminal chiasmata were observed and homologous chromosomes held together at three chiasma points were also not of very infrequent occurrence (Fig. 7). Open bivalents in contrast to closed ones were observed to be very few in number. A study of the chiasma frequency and terminalization coefficient in all the strains is expected to yield significant data. Distinct seven bivalents were clearly observed during diakinesis as well as metaphase stages of first meiotic division (Fig. 8). Anaphasic segregation though was found to be more or less normal (Fig. 9), rare occurrence of inversion bridges resulting in a dicentric chromosome and a fragment were noted in the strains IP: 1, IP: 9 and IP: 14. No case of lagging or non-disjunction could be encountered in any of them.

In the second division, too, the chromosomes were found to behave in a fairly regular manner. Clear seven and seven chromosomes could be counted in the two

spindles of the second meiotic metaphase (Fig. 10). Their segregation, too, was found to be normal, resulting in the regular formation of tetrads. Counts of morphologically abortive pollen grains showed the sterility to be 2-3%.

Difference in karyotypes of the strains studied :

It is clear from the above that the karyotypes of these different strains, although similar in the gross morphology of the chromosomes, differ in minute structural details from one another, particularly with respect to chromosomes with secondary constrictions. It has already been pointed out that the number of secondary constrictions in case of the strains IP: 14, IP: 20 and IP: 24 is ten, in IP: 1 fourteen, in IP: 9 sixteen and in IP: 13 eight.

For the sake of convenience in comparison, the chromosome types A, B, C, D, E, F and G of the strains IP: 14, IP: 20 and IP: 24 have been taken to be the standard ones and the chromosome types of the other strains in relation to their difference with these standard ones have been discussed below (Fig. 11).

The type 'A' with three secondary constrictions is present in all the strains studied.

The type 'B' with one secondary constriction is absent in the strain IP: 13, where it has been replaced by a chromosome pair with submedian primary constrictions G_1G_1 similar to the type G. In the strains IP: 1 and IP: 8, it has been replaced by B_1 type, a pair of chromosomes having morphology more or less similar to the type B, but differing in having an extra constriction at the end of the long arm.

The type 'C' with one secondary constriction is present in all the strains studied.

The D, E and F pairs of chromosomes belonging to one type are present in the strains studied, excepting the strain IP: 1, where one of the pairs, say, for instance, D, is replaced by G pair type, a pair of chromosomes having morphology similar to the type C, with submedian primary constriction and having a satellite at the end of the short arm.

The 'G' type of chromosomes is present in all the strains studied, excepting the strain IP: 9, where it has been replaced by B_2 type, a pair of chromosomes having morphology similar to the type B_1 , with submedian primary constriction, a secondary constriction very near the primary one towards the shorter arm and a satellite at the end of the long arm.

DISCUSSION

The chromosome counts of different species of *Hordeum*, as reported by previous authors, show a basic number of seven. Different polyploid species have been found, both in nature as well as in cultivated forms. Chin's (1941) observation on the meiotic behaviour of different species of the genus shows that both auto- and allopolyploidy have played an important rôle in the evolutionary process of these species. Tetraploid forms of *H. spontaneum* Koch., *H. jubatum* L., *H. gussonaeum* Parl., and *H. murinum* L., as studied by him, showed regular occurrence of bivalents during meiosis, indicating their possible allopolyploid nature. On the other hand, frequent formation of multivalents in the tetraploid form of *H. bulbosum* L. suggests its origin, possibly through autopolyploidy.

Lewitsky (1931) reported that all cultivated diploid species of *Hordeum* possess two pairs of chromosomes with satellites. Chin (1941) also corroborated his observation. The occurrence of one pair of satellited chromosomes in the wild diploid, and two pairs in the wild tetraploids, and the presence of two pairs of satellited chromosomes in the cultivated diploids, led the latter author to suggest that the phylogenetic relationship between the wild and cultivated species might not be close. Such a suggestion, however, in the light of our present-day knowledge of

cytogenetics seems no longer tenable. The fact has now become well established that, apart from polyploidy, there are other ways by means of which an increase in the number of nucleoli and satellites in a species may take place.

Oinuma (1952) has recently shown that cultivated barleys are derived from wild ones and the structural changes in the chromosomes involved have also been worked out. Quite a large amount of literature (Bhaduri, 1942a) has accumulated in the last few years showing that interchanges involving nucleolar and non-nucleolar chromosomes may result in an increase in the number of constrictions and nucleoli of a species. Recent works of Bhaduri and Bose (1947) on different members of the family *Cucurbitaceae* and of Chakravarty (1948) on different species and genera of *Scitamineae* have brought about important evidences in support of the fact that fragmentation of chromosomes also plays an important rôle in bringing about a high number of nucleoli and satellites of the living nuclei. All these recently accumulated data suggest that the earlier assumption of Chin, regarding the phylogenetic relationship between the wild and cultivated species of *Hordeum*, may not necessarily be valid.

In the present investigation, although in one of the strains, viz. IP: 13, two pairs of chromosomes with secondary constrictions have been noted, additional supernumerary constrictions are present, making the total number of secondary constrictions and satellites to be eight. In the strain IP: 9, number of constrictions excluding the primary ones are sixteen, present in four pairs of chromosomes. In the strain IP: 1, fourteen secondary constrictions and satellites are distributed in three pairs of chromosomes. In the other strains, ten constrictions are distributed in three pairs of chromosomes. It is, therefore, apparent that the number of secondary constrictions and satellites, present in seven pairs of chromosomes in diploid species of *Hordeum*, is much higher than that noted by previous workers. The possibility of all these constrictions being nucleolar in nature is not precluded, because of the presence of a high number of nucleoli, as many as ten, in the somatic cells of at least one of the strains. In any case, this aspect of study, which has not been dealt with, is yet to be done.

Examination of wild varieties of barley as done to some extent by Oinuma (1952), employing critical technique from which the cultivated strains have evolved, may reveal whether these high numbers of constrictions were originally present in the wild complement or their evolution has taken place through structural changes of chromosome. A thorough search in this direction is highly desirable.

As has been pointed out in the text, definite evidences have been obtained to show that many of the strains of cultivated barley differ from each other with respect to their karyotypes. It is not unlikely that structural interchanges are directly responsible for the different karyotypes amongst the different cultivated strains which differ from each other in minor morphological characters only. It is interesting to note that Oinuma (1952) has obtained intervarietal hybrids in which the presence of interchanges or rings in meiosis has been detected.

The absence of any ring formation and the occurrence of regular bivalents during meiosis in these strains are probably due to their homozygous and stable nature produced as a result of continuous cultivation and judicial selection. Rare occurrence of inversion bridges, too, points towards the possibility of structural changes of chromosomes playing an important part in the evolution of these strains. In other strains, where such bridges have not been encountered, it is not at all unlikely that the absence of bridges is not due to the absence of any inverted segment in the chromosome, but due to the homozygous nature of the strain concerned, where such inversions are present in both the homologues.

If the above conclusion is correct, then the occurrence of rings of chromosome, instead of regular formation of bivalents during meiosis, is expected in intervarietal hybrids of *H. vulgare* L., where the homologous segments in otherwise non-homologous chromosomes will have the chance to meet each other. The

report of Oinuma (1952) already mentioned is noteworthy in this regard. Once this theory is established, extensive hybridization of different strains of *Hordeum vulgare* would provide good materials for the study of identification and classification of chromosome ends of barley as has been done in case of *Datura* by Bergner *et al.* (1933), *Zea* by McClintock (1934), *Tradescantia* and *Rhoeo* by Bhaduri (1942a, 1942b). It is worth noting that Hagberg and Tjio (1950) have recently succeeded in getting artificial mutants of barley showing translocations.

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SUMMARY

1. A karyotype analysis of six different strains of barley, raised in the Indian Agricultural Research Institute, New Delhi, has been carried out. It has been shown that all the strains, though having the same number of chromosomes, i.e. fourteen, more or less similar in gross morphology, differ with respect to the number of nucleolar constrictions present in the complement.

2. It has been found that the presence of a high number of supernumerary constrictions in the chromosome complement is a constant feature of all the strains studied. A high number of nucleoli has been observed in root tip cells of some of the strains. This is in strong contradiction with the report of earlier workers who reported only two pairs of satellited chromosomes in the cultivated diploid species of *H. vulgare* L. All these details in chromosome morphology could only be brought about by the application of special technique, viz. platinic chloride and formalin mixture with an increased proportion of formalin as the fixing fluid and a prefixation treatment in colchicine solution.

3. Meiotic behaviour of all the strains studied showed more or less regular behaviour excepting the rare occurrence of inversion bridges in some of the strains.

4. It has been suggested that a critical investigation with the application of the improved technique, as adopted here, should be made of the wild diploid species of *Hordeum* to find out whether these high number of constrictions were present in the original diploid set or the evolution has taken place through structural interchanges from the original set.

5. The regular behaviour during meiosis has been claimed to be due to their stable homozygous nature, produced as a result of continuous cultivation and judicious selection. In view of the fact that formation of rings during meiosis are expected in intervarietal hybrids where homologous segments in otherwise non-homologous chromosomes would get the chance of meeting each other, it has been suggested that extensive hybridization of different varieties of *H. vulgare* L. is necessary to have good materials for the study of classification and identification of chromosome ends in barley.

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THE ATRIOVENTRICULAR BUNDLE IN THE HEART OF THE BANDED KRAIT, *BUNGARUS FASCIATUS* *

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INTRODUCTION

His (1893) discovered and described a muscular bundle connecting the atrial and ventricular septa in the adult mouse, new-born dog, and new-born and adult man. Keith and Flack (1906), Kistin (1949) and Prakash (1954*a*) have pointed out that the bundle of His is the only tissue to connect the atria and the ventricles of the heart of mammals. Kent (1893) studied the heart of a large number of mammals but could not find any specific bundle to connect the atria and the ventricles. He stated that in mammals like all other vertebrates multiple muscular connexions exist to transmit the atrial wave of contraction to the ventricles. Davies (1930) believes that, correlated with the rapid rate of heart beat, birds possess the bundle of His as well as the multiple muscular connexions of Kent for a quick transmission of the cardiac stimulus of contraction from atria to ventricles. Prakash (1954*d*) holds the view that, if any atrioventricular connexion in addition to the bundle of His would be present in mammals, premature excitation of the ventricle would occur giving rise to Wolff-Parkinson-White syndrome.

Regarding the phylogeny of the conducting system of the heart of vertebrates, Davies (1930) believed that the atrioventricular conducting tissue of the bird's heart presents an arrangement which is intermediate in nature between that of fish and reptile on one hand and that of mammal on the other. However, Davies, Francis and King (1952) were unable to find any specialized tissue in the crocodilian heart and therefore they believe that the impulse initiating and conducting tissue of the heart of birds and mammals is neomorphic in nature. Prakash (1953, 1954*a*, *b*, *c*, *d*) upholds the view that the specialized conducting system of the heart of birds and mammals is not a neomorphic development but is a further specialization of a similar system which is present in the heart of lower vertebrates.

Having observed special impulse initiating and conducting structures in the heart of fishes and amphibians (Prakash, 1953, 1954*b*, *c*), the object of the present study was to find out if the reptilian heart also possessed these structures or not. With this aim in view, in the present investigation the heart of the banded krait, *Bungarus fasciatus*, has been chosen and studied with special reference to its atrio-ventricular connecting tissue.

MATERIAL AND METHODS

About a dozen eggs of *B. fasciatus* were collected from the area adjoining the small lake of Bhopal. After breaking the mature eggs, young ones of kraits were removed and dissected to take out the hearts in beating condition. The hearts

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were immediately fixed in Bouin's picroformol. As usual serial sections, six micra thick, were cut of paraffin embedded blocks, and stained in acid fuchsin.

OBSERVATION

The two atria almost of equal size communicate with a single ventricle. A spherical body composed of fine narrow interlaced muscle fibres has been observed in the serial sections of the heart at the atrioventricular junction (Figs. 1, 2). This body lies as an isolated bundle and is quite distinct from the tissue present all round it. The cells of the bundle, as well as the fine narrow muscle fibres which form these cells, take a deep stain indicating the specialized nature of the bundle. A definite shape has been assumed by this bundle, because of the presence of a compact layer of fibres all round it (Fig. 3). An examination of the sections passing through this bundle, under Reichert's Fibroscope, has revealed that the fibres from the left atrium enter into the cranial portion of the bundle, while the ventricular fibres are continuous with the muscle component present in the caudal part of the bundle. The fibres of the bundle resemble on one hand those of the atria and on the other those of the ventricle. The structure and disposition of the bundle, and the fact that the atria and the ventricle are connected with each other only through this bundle, warrant its identification as the atrioventricular bundle.

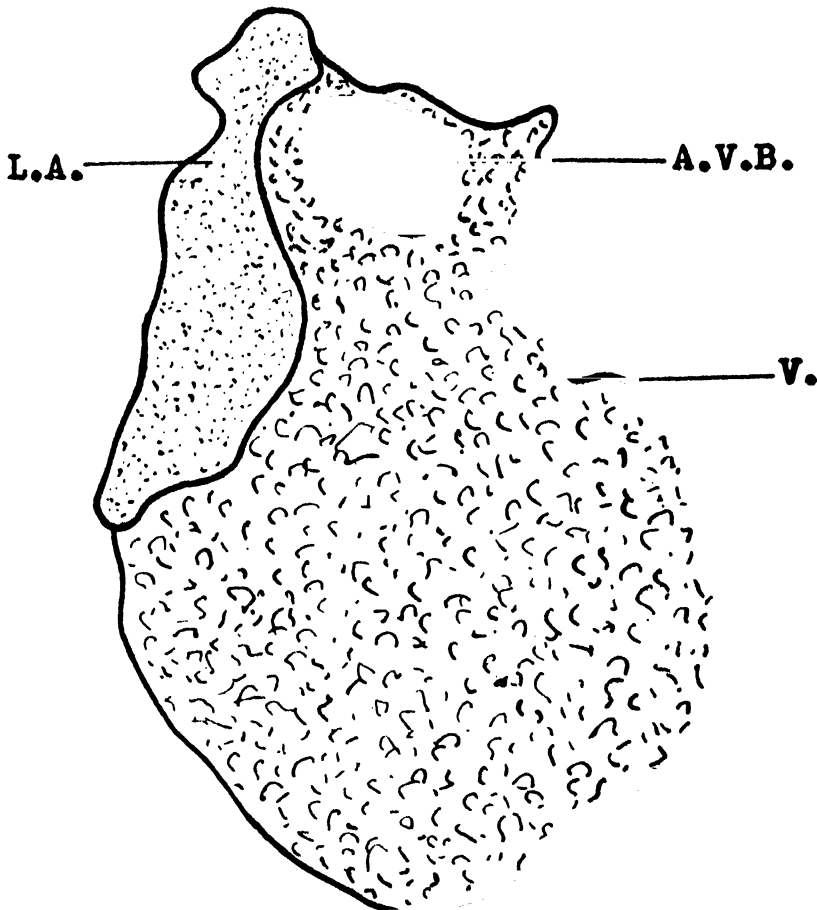


FIG. 1. Diagram to show the position of the atrioventricular bundle in *Bungarus fasciatus*.
× 500.

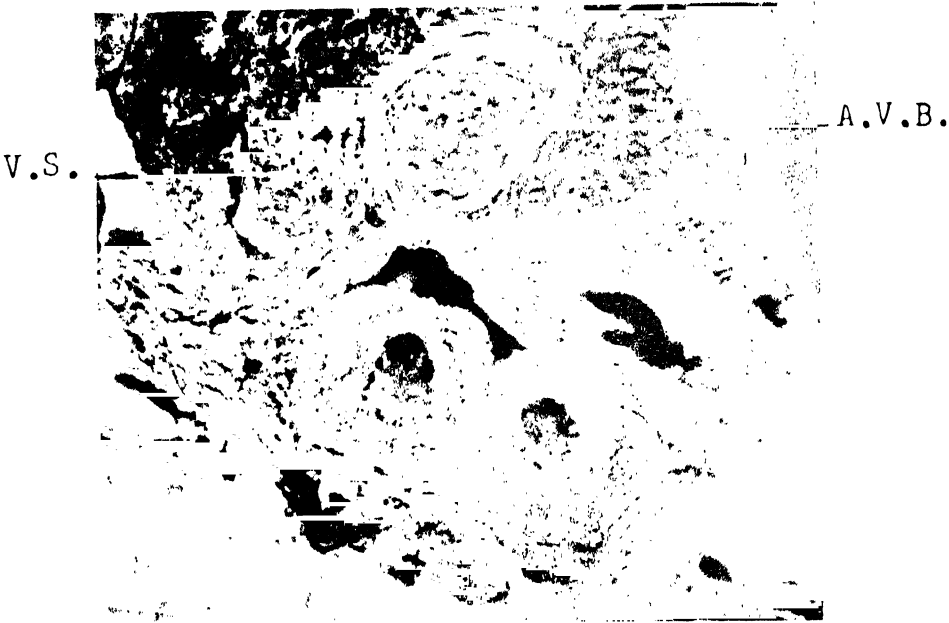


FIG. 3. Photomicrograph of a section passing through the atrioventricular bundle of *Bangarus fasciatus*. 500.

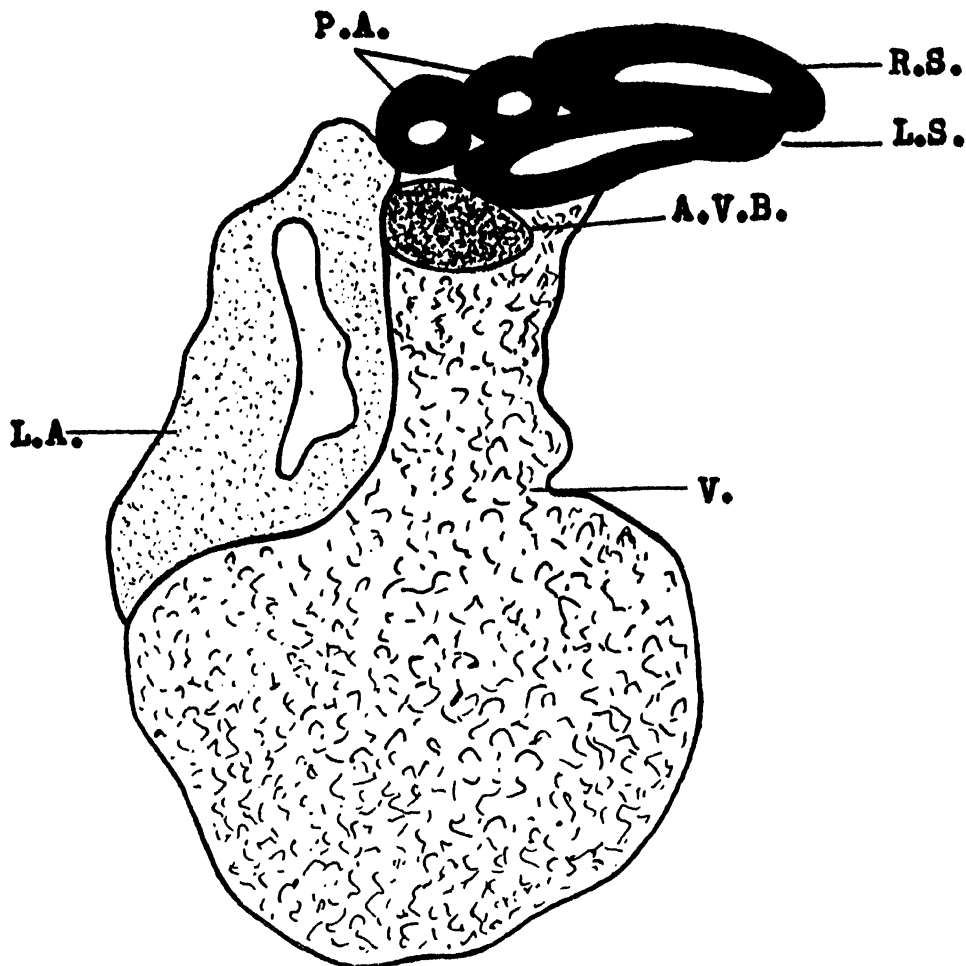


FIG. 2. Diagram to show the atrioventricular bundle at the junction of the left atrium and the ventricle of *Bungarus fasciatus*. $\times 500$.

A continuity between the atrial and ventricular muscular fibres through the muscle component of the atrioventricular bundle, and the fact that this bundle is the only tissue to connect atria and ventricle show that the muscle tissue forms an integral part of the impulse conducting system of the heart of the kraits.

DISCUSSION

In the heart of the banded krait, *B. fasciatus*, an atrioventricular bundle, resembling the atrioventricular plug of fishes and amphibians (Prakash, 1953, 1954c) and the bundle of His of birds and mammals, is also present. It connects the left atrium with the ventricle for the propagation of the cardiac rhythm of contraction. There is, therefore, no other connexion between the atria and the ventricles than that through the atrioventricular bundle which passes on the atrial stimulus of contraction to the ventricle. As this bundle is formed of special muscle fibres, it is to be inferred that the impulse conduction is carried out by the muscle tissue. The myogenic theory of cardiac conduction, therefore, holds good also in reptiles as in other vertebrates (Prakash, 1954; Davies and Francis, 1946).

Robb (1953) observed 'Purkinje-like' specialized tissue in the heart of the turtle, *Pseudomyia elegans*. Mori (1955), in his paper on the atrioventricular system of the crocodile heart, stated that, 'the connecting system in the inter-ventricular septum in crocodile heart is the precursor of the principal connecting system of bird and the conducting system of mammalia'. Prakash (1953, 1954c) has also observed that an atrioventricular plug which is a bundle of closely woven fibres is present in the heart of the fish, *Heteropneustes fossilis*, and in the tadpoles of the frog, *Rana tigrina*, to transmit the contraction impulse from the atria to the ventricle. The present study reveals that in the heart of *B. fasciatus* a similar atrioventricular bundle is also present to conduct the stimulus of contraction from the atria to the ventricle. From the above it is concluded that the plug of fishes and amphibians and the atrioventricular bundle of reptiles resemble in structure, and presumably in function also, the atrioventricular bundle of birds and mammals.

SUMMARY

In the heart of the banded krait, *Bungarus fasciatus*, an atrioventricular bundle is present to conduct the stimulus of contraction from the atria to the ventricle. Furthermore, it is observed that the atrioventricular bundle of the heart of krait resembles in structure and presumably in function also the atrioventricular plug of the heart of fishes and amphibians and the bundle of His of the heart of birds and mammals.

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ABBREVIATIONS

A.V.B.—Atrioventricular bundle; L.A.—Left atrium; L.S.—Left systemic arch; P.A.—Pulmonary artery; R.S.—Right systemic arch; V.—Ventricle; V.S.—Ventricular septum.

STUDIES ON THE BIOLOGY OF THE INDIAN FRESH-WATER EEL, *ANGUILLA BENGALENSIS* GRAY

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CONTENTS

	<i>Page</i>
Introduction	259
Length-weight relationship and condition factor	260
Food and feeding habits	263
Immigration of elvers into estuaries	266
Age and growth	
(a) Growth of elvers during the first year of their life in fresh water	267
(b) Scales as indicative of age and growth of the adult eel	269
(c) Length frequency	276
Summary	277
Acknowledgements	278
References	278

INTRODUCTION

The Indian fresh-water eel, *Anguilla bengalensis*, is widely distributed in the Indian sub-continent, though it is 'much rarer on the hills than in the plains' (Day, 1878). Though eels are generally acclaimed as very important food fish in many of the European and in certain Asian countries, they are seldom eaten in India except by the poorer classes. However, in certain parts of South India, the eels are relished and fetch a much better price than carps.

Though extensive researches have been conducted on various aspects of the biology of the eel in the European countries, practically no work has been done on any of the Indian species (viz. *A. bengalensis* and *A. bicolor*), except for certain observations of a preliminary nature by Rahimullah *et al.* (1944). Schmidt (1932) recorded the occurrence of leptocephali of *A. bengalensis*, in the Mantewi Deep, west of Sumatra.

The present account is based on the study of over 3,500 specimens of this fish (ranging from 40–700 mm.). Most of the specimens were collected from an escape channel of the Calcutta Waterworks at Pulta near Barrackpore, which is situated at a distance of nearly 100 miles from the mouth of the River Hooghly. The escape channel maintains its connection with the river all the year round and gets flooded with river water during high tides. The eels were generally collected with the help of small-meshed drag nets, but many of the bigger specimens were caught by hand from holes and crevices under the banks. Regular fortnightly collections were made commencing from January, 1952 to March, 1953.

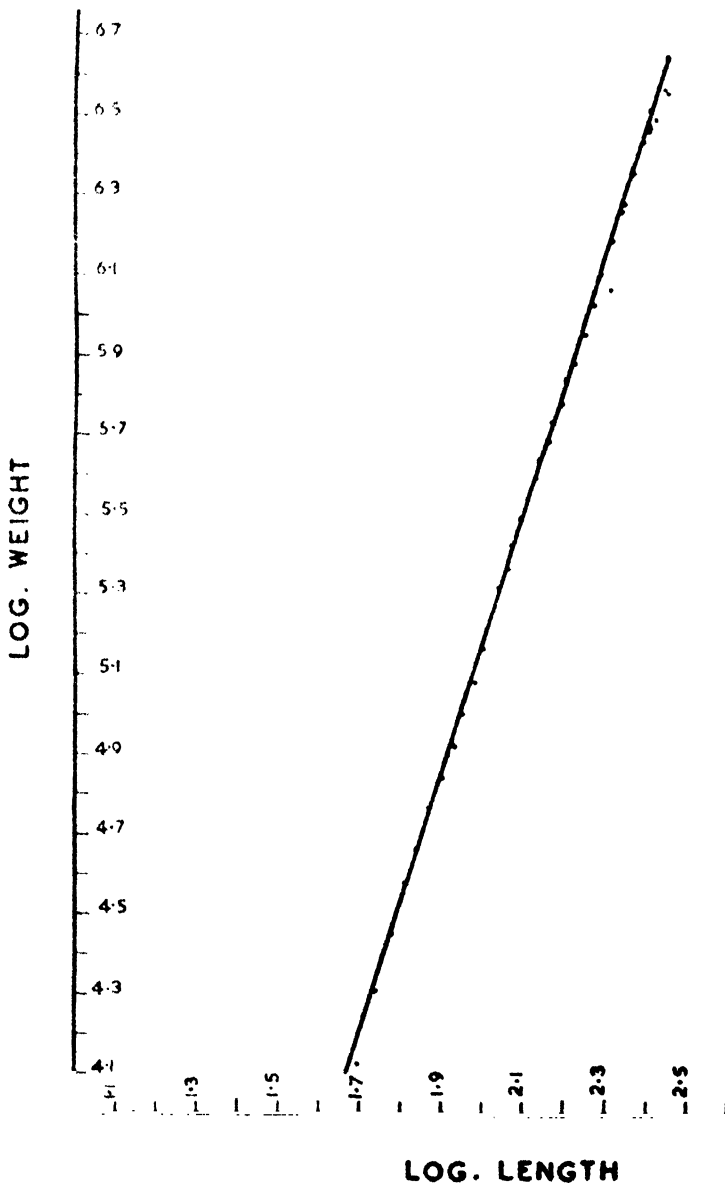
To ascertain the period of immigration of newly metamorphosed leptocephali (glass eels), data were collected on their seasonal occurrence in the Hooghly estuary, by making regular weekly collections from two *Bhinjals* (bag nets with close meshed cod ends) throughout the year 1952.

Regular tow-net collections made in the coastal waters of the Burhabulong estuary at Chandipore (Orissa) to obtain leptocephali of *A. bengalensis* proved futile, though larvae of various other genera of Apodes, viz. *Muraenids*, *Muraenesocids*

and *Ophichthyids*, were obtained (Jones and Pantulu, 1952 and 1955; Pantulu and Jones, 1954).

LENGTH-WEIGHT RELATIONSHIP AND CONDITION FACTOR

The length-weight relationship of specimens of *Anguilla bengalensis* ranging from 50-300 mm. in total length was studied. Since the sexes were not apparent in the specimens they were not considered separately. All specimens less than 140 mm. in length were weighed to the nearest milligramme in a physical balance, and those above that length were weighed to the nearest $\frac{1}{2}$ gramme in a trip-type of pan balance. The mean weight of each 5 mm. length interval was calculated and its log. was plotted against the log. of the mid point of the length interval. The regression was found to be a straight line (Text-fig. 1).

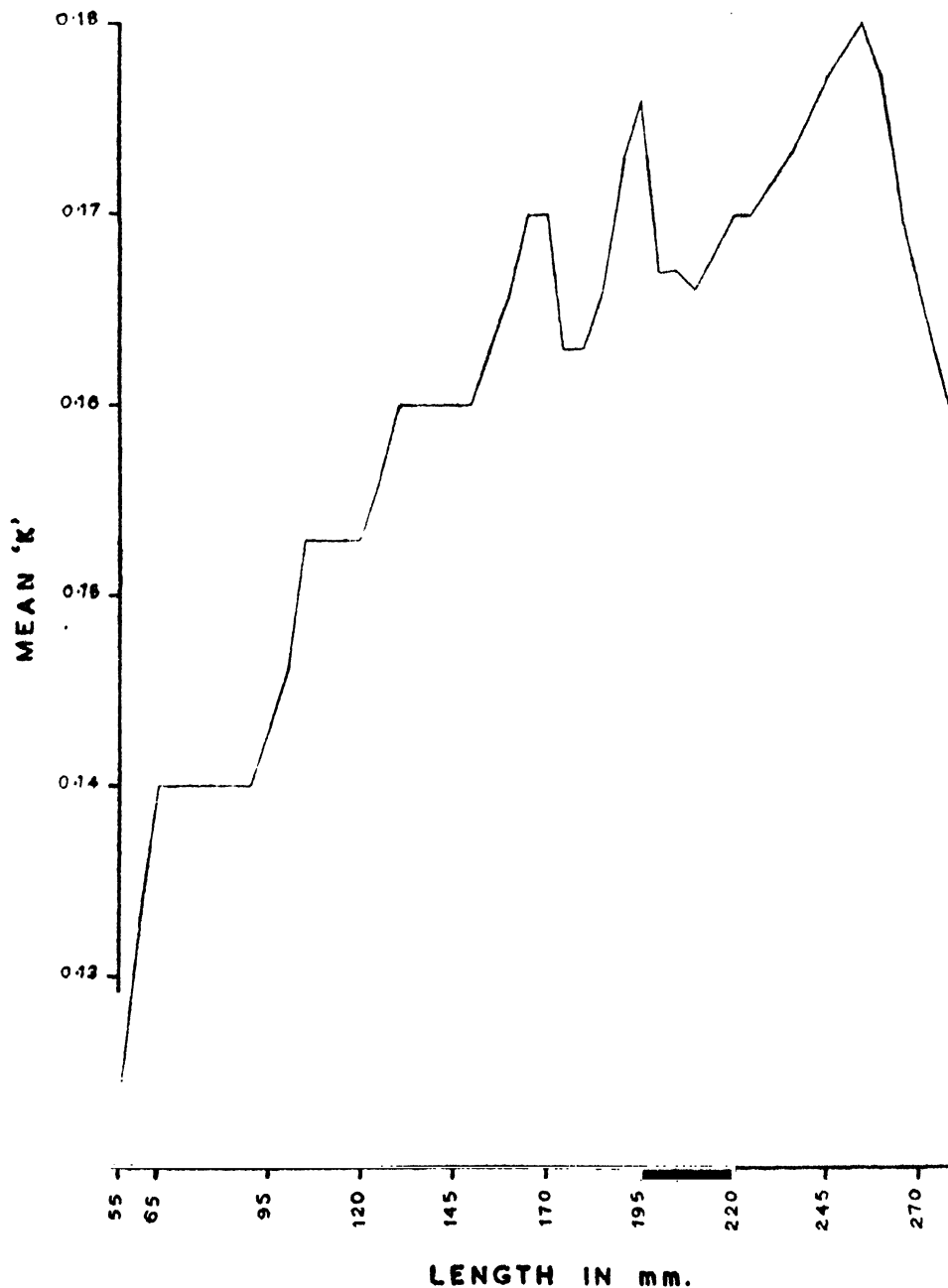


TEXT-FIG. 1. Logarithmic relation of weight and length of *Anguilla bengalensis*.

An analysis of the length-weight data showed a very high correlation ($r = 0.91709$) and an analysis of variance also indicated that the regression was highly significant.

Length-weight relationship of the fish was determined from the general formula $W = CL^n$, in its logarithmic form, viz. $\log. W = \log. c + n \log. L$ (where W = weight in grams; L = length in mm. and c and n are constants). Values of c and n were calculated to be as under:—

$$n = 3.20265 \text{ and } \log. c = 2.75739$$



TEXT-FIG. 2. Mean 'K' at different lengths of *A. bengalensis*.

The length-weight relationship of the species may be expressed by the formula

$$W = 0.057199 \times 10^{-5} \times L^{3.20265}$$

Condition factor.—The condition factor K was calculated from the formula

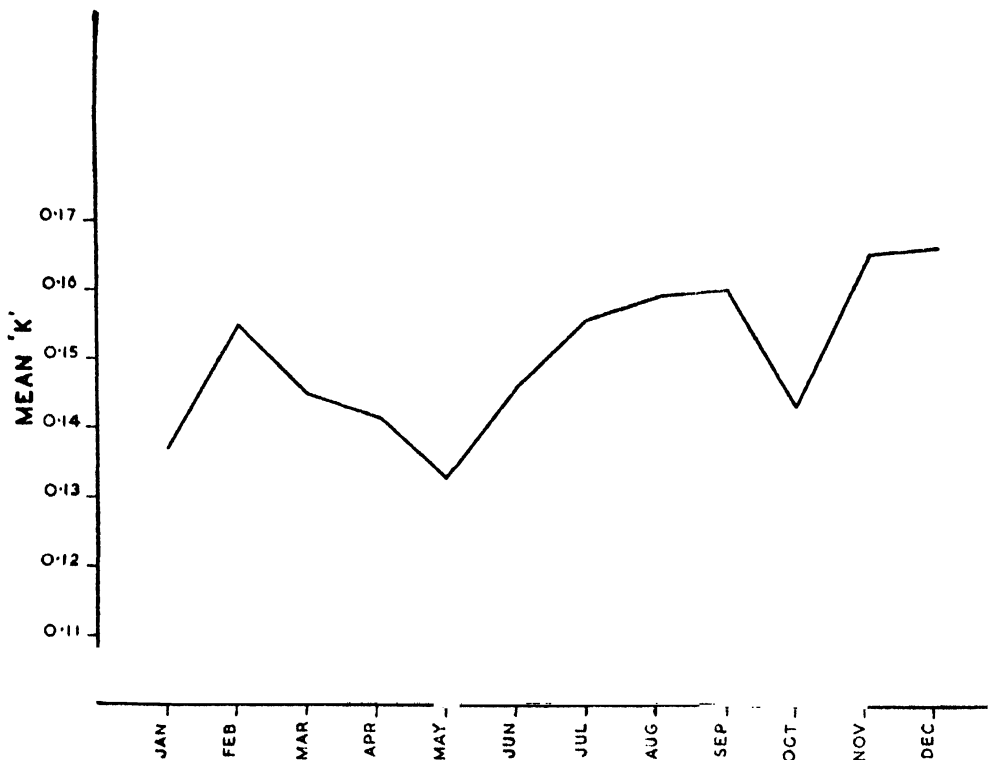
$$K = \frac{W 10^{-5}}{L^3}$$

where K is the condition factor, W is the weight in grammes and L is the length in millimetres.

K values were calculated separately for each 5 mm. length group of eels collected during the whole year and for all length groups combined for the different months of the year. The values are delineated in Text-figs. 2 and 3.

From Text-fig. 2 it appears that K increases with the increase in length of fish from 50–165 mm. There are sudden, marked falls in the values at about 170, 195 and 255 mm. The reasons for these inflexions are rather obscure, considering that at these lengths there is neither indication of transition from one stage in the life history to another (such as yellow to silver eels), nor evidence of increased metabolic strain. It is, however, interesting to note that the mean lengths of fish having one and two rings on the scales (*vide* page 274) are 183.56 and 249.50 mm. respectively. Therefore the inflexions at the first and last points (i.e. 170 and 255 mm.) may probably be attributed to the metabolic strain which leads to the formation of rings on scales.

No definite trend could be observed in the K values for different months of the year except that, in general, the fish captured in the latter half of the year appear to have a higher condition factor than those obtained in the earlier half (Text-fig. 3).



TEXT-FIG. 3. The monthly fluctuations in the 'K' values of *A. bengalensis*.

FOOD AND FEEDING HABITS

The results of the study on the food habits as revealed by the analysis of gut contents of nearly 450 specimens ranging between 140–660 mm. (collected during various months of the year 1952) are discussed below. As it is generally believed that eels feed during night, the specimens were collected during the early hours of the morning.

The volumes of the different food items were determined by displacement method correct to the nearest 0.05 c.c. Food organisms were identified up to species wherever possible or only up to groups where, due to their being partially digested, further identification was rendered impossible.

The principal groups of food organisms found were: annelids, crustaceans, fish, insects and macro- and microphytes. Other items which were found only rarely, and the consumption of which was regarded as accidental, were listed under miscellaneous organisms. A detailed list of the organisms encountered among the gut contents is given in Appendix I.

Considering the volume of individual groups of food organisms, in proportion to the total volume of food consumed during the entire period (i.e. Jan.–Dec., 1952), it was found that fish predominated as a single item of food, accounting for 40.34%; crabs ranking second accounted for 26.04% and prawns 20.60%. The relative predominance of the rest of the organisms, viz. insects, megalopa larvae of crabs, macro- and microphytes, annelids and miscellaneous items accounting for 4.24%; 2.92%; 2.26%, 2.01% and 1.53% respectively, was comparatively negligible.

Judged also by the frequency of occurrence of various organisms in the total number of stomachs examined (*vide* Table I), fish formed the most important item of diet, prawns ranking as a close second and megalopa larvae, third.

TABLE I

Frequency of occurrence (in percentage) of various food organisms present in the stomach contents

Fish	23.55
Prawns	22.83
Megalopa larvae	14.86
Crabs	14.50
Macro- and microphytes	6.88
Insects	6.88
Miscellaneous	5.43
Annelids	5.07

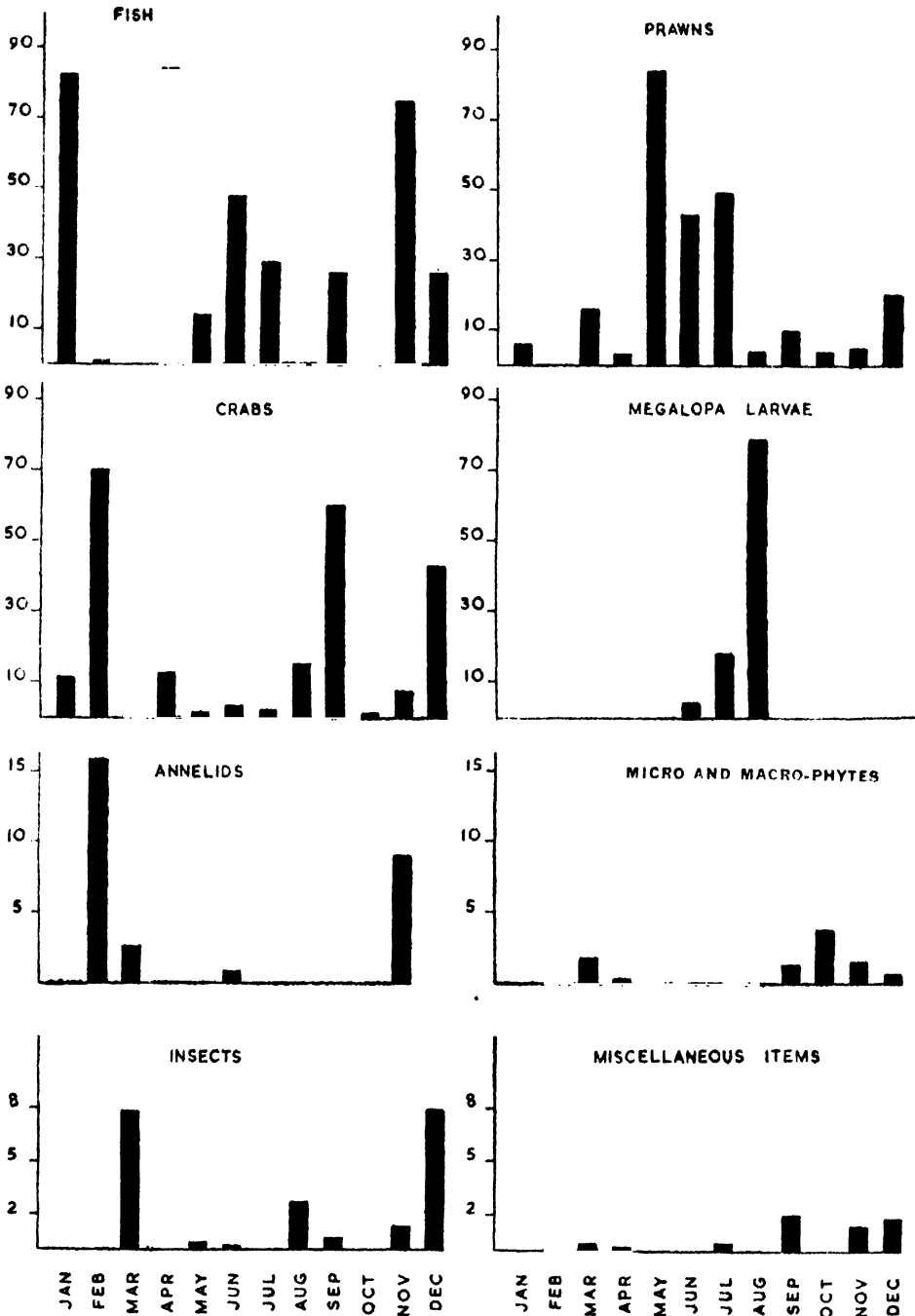
Fish, as already mentioned, form the principal food of the eel and occur among the stomach contents almost throughout the year, excepting in the months of March and August. They are most predominant in January, April and October. Fry and fingerlings of important species like *Hilsa ilisha*, *Setipinna phasa*, *Pama pama*, etc., are found to have been consumed by the eel. *Anguilla* is believed to have cannibalistic tendencies, but in the present investigation it was observed that though large numbers of small elvers abound in the environment all the year round, and particularly during January and February, only in two cases elvers were found among the stomach contents.

Prawns and crabs ranked next to fish in importance as food of eels. Prawns were particularly predominant in the stomach contents in the months of May, June and July, whereas crabs were more common during February, March and September.

Megalopa larvae were consumed in large numbers during the month of August when enormous numbers of these larvae occur in the river; 78% of the fish examined in the month were found to have fed only on these organisms.

TABLE II
Percentage composition of stomach contents

Food items	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Fish ..	82.56	1.54	..	84.09	13.8	48.34	29.31	..	25.81	91.12	75.38	25.78
Prawns ..	6.42	..	16.37	2.87	84.11	42.7	49.52	3.7	10.22	3.62	4.61	19.92
Crabs ..	11.07	70.66	70.93	12.45	1.12	3.56	2.22	14.79	39.68	1.32	7.69	42.89
Megalopa larvae of crabs	0.37	4.20	18.55	78.77
Insects ..	0.13	..	7.88	0.1	0.45	0.19	..	2.74	0.75	..	1.4	8.98
Annelids	14.59	2.63	..	0.15	0.75	7.69	..
Micro- and macrophytes	..	11.67	1.75	0.24	..	0.07	0.04	..	1.40	3.95	1.54	0.46
Miscellaneous organisms	..	1.54	0.44	0.34	..	0.19	0.37	..	2.15	..	1.54	1.95



TEXT-FIG. 4. Histograms showing the monthly variations in the percentage composition of the different items of food eaten by *A. bengalensis*, during different months.

. Among the insects found in the gut contents, in addition to aquatic larvae of Coleoptera, Trichoptera and Diptera, were several terrestrial insects like *Gryllo-talpa*, de-elated termites and ants, which were probably captured when they were accidentally washed into the current.

Among micro- and macrophytes found in the stomachs, were green algae, blades of grass, roots and seeds of plants and aquatic weeds, but the quantity of these items, and the frequency of their occurrence in the guts, were negligible. Fresh-water oligochaetes formed the bulk of the annelids consumed by the eel. Most of these organisms were found in a fairly advanced state of digestion, rendering their generic or specific identification impossible. In addition to the above, centipedes and spiders were also found, though rarely, among the stomach contents. Pebbles and sand also occurred in the stomachs. The occurrence of a wide variety of organisms, including those foreign to an aquatic habitat, shows a predominantly omnivorous feeding habit.

IMMIGRATION OF ELVERS INTO ESTUARIES

Newly metamorphosed elvers, which are termed 'glass eels', are transparent, and totally unpigmented. Their lengths range between 40 and 58 mm. with a mean length of 50.9 mm.

From the analysis of data relating to the catch of glass eels, it was observed that they occurred in the estuary only during the period October to March. During the months October, November, December and March, fewer specimens were obtained than during January and February (*vide* Table III). This indicates that immigration of elvers into the estuary after metamorphosis takes place during the colder months of the year, commencing from October and continuing till the end of March following, the peak period of immigration being January and February.

TABLE III

Occurrence of glass eels in the Hooghly estuary

Month			Average number of elvers obtained per net	Length range in mm.
January, 1952	64	50-58
February, 1952	80	47-57
March, 1952	20	48-51
April, 1952
May, 1952
June, 1952
July, 1952
August, 1952
September, 1952
October, 1952	16	47-51
November, 1952	8	48-50
December, 1952	4	40-50

Corroborative evidence for the above observations was obtained, from the occurrence of glass eels, in the regular fortnightly collections made from the escape channel at Barrackpore, where also glass eels were available only during the months October to March.

It is interesting to note that in the case of elvers of European eels also 'invasion (into estuaries) begins in November or December and reaches its height in January, February and March' and the tiny fry measure 'on an average two to three inches in length' (Roule, 1933). Gemzoe (1908) states that the 'date of immigration falls in spring and may with approximate accuracy be placed at May 1'. Petersen (1894) observed that small eels, about 70 mm. in length, wander into fresh waters

of Denmark in large numbers, in spring and early summer, and he places the time of early immigration in the months March to June. Johansen, as quoted by Gemzoe (*op. cit.*), concludes that glass eels abound in watercourses in spring (March and April) and have lengths of 70 to 72 mm.

AGE AND GROWTH

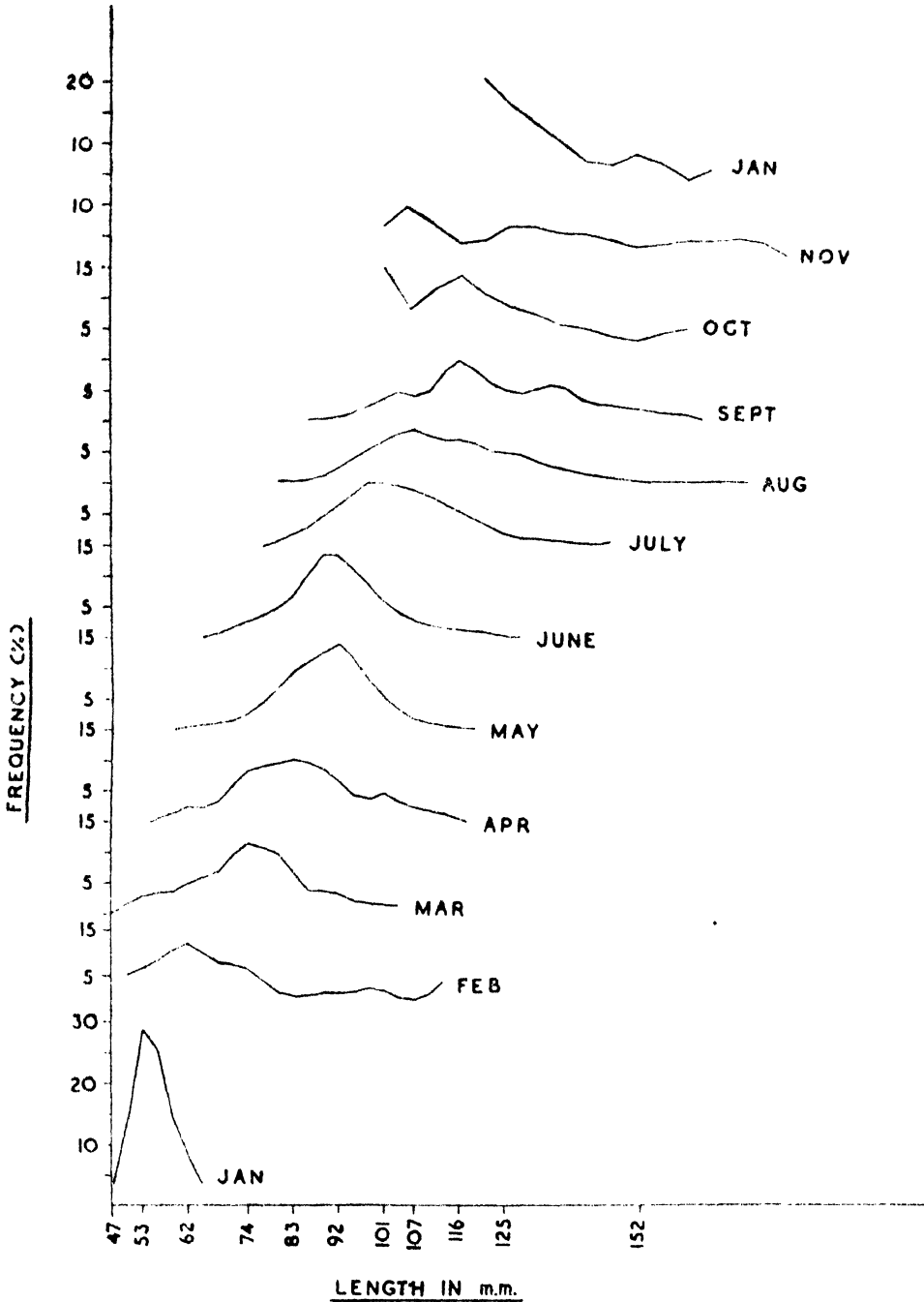
(a) *Growth of elvers during the first year of their life in fresh water*

To ascertain the growth of elvers during the first year of their life in inland waters, regular fortnightly collections were made from January, 1952 to January, 1953 (*vide* page 259). Figures for the month of December, 1952, were left out of reckoning, since the sample was considered inadequate. In Text-fig. 5 are given the smoothed length frequency curves for the various months which, except in a few cases, are found to be unimodal. In January, 1952, the mode is seen to be at 53.0 mm., while in February the mode is at 62.0 mm. On the reasonable assumption that the population has been the same throughout, the progression of the mode by 9.0 mm. is considered to indicate the growth during the month. In February, however, there is another small mode at 98.0 mm., which probably represents the size group of the individuals which migrated into the estuary earlier in the season (i.e. since October, 1951). In March, this second peak is no longer visible, but another peak, representing individuals smaller than the main February modal size, is discernible, due obviously to the presence of smaller size groups as a result of continued recruitment. The modes in May and June are in the same positions and the absence of any difference in the modal values of these two months may be attributed to the fact that in samples collected in May there is a predominance of specimens collected in the second fortnight, whereas in June samples the catches of the first fortnight were dominant. A similar condition is seen in the modal values for September and October which coincide, though the reasons for the non-progression of the mode in this case are obscure, with the number of specimens in the samples collected during different fortnights of the two months being nearly the same. The modal values in different months of the year and growth from month to month are given in Table IV.

TABLE IV

Growth in length of the elvers of A. bengalensis during the first year of their life in inland waters

Month	Size range in mm.	N	Modal values	Increment in length in mm.
January, 1952 ..	47- 65	46	53	..
February, 1952 ..	50-113	134	62	9
March, 1952 ..	47-104	271	74	12
April, 1952 ..	56-116	192	83	9
May, 1952 ..	59-119	691	92	9
June, 1952 ..	65-128	615	92	..
July, 1952 ..	77-146	358	101	9
August, 1952 ..	80-173	543	107	6
September, 1952 ..	86-164	201	116	9
October, 1952 ..	101-161	40	116	..
November, 1952 ..	91-181	46	126	10
December, 1952 }	..
January, 1953 ..	121-171	34	161 }	25



TEXT-FIG. 5. Length frequency curves of elvers of *A. bengalensis* collected during different months, from the Hooghly estuary.

As shown in the above table, the elvers attain a length of about 151.0 mm., or register a growth of about 98.0 mm. at the end of the first year after immigration into estuaries.

Having collected a large number of elvers of *A. bengalensis* about 6 inches (152.0 mm.) long at Nizamsagar dam, Rahimullah *et al.* (1944) stated, 'we are of the

opinion that most probably it (*A. bengalensis*) breeds in fresh water and has left the habit of going to the sea'. This opinion was based on the assumption that the elvers collected were too small and too young to travel a distance of 450 miles (from the sea to Nizamsagar dam), particularly after negotiating the anicuts at Rajahmundry and Dummugudem, a precipitous ascent at Bhadrachalam and swift currents in the river in a rocky territory *en route*. From the present investigation it is clear that 6 inch elvers should be about a year old. On the reasonable presumption that immigration into the Godavari estuary takes place in the same months as into the Hooghly, the elvers collected in Nizamsagar in September, 1943 (by Rahimullah and others) could be considered to have commenced their journey upstream after metamorphosis some time early in 1942. Hence it is not incredible that these elvers could have travelled a distance of 450 miles, in a period of over one year. Further, eels are known to negotiate obstacles of the type considered insurmountable to them by Rahimullah *et al.* Cairns (1941) observed, 'eels are capable of climbing vertical walls provided the surface is damp. The eels go up the highest and steepest water-falls in the country by travelling up the damp sides out of the main rush of water. During migrations they will travel across land in heavy rains or if grass is wet with dew'. In the light of the above facts and considering that no glass eels were obtained at Nizamsagar, it is felt that sufficient valid reasons are lacking to support the assumption of Rahimullah *et al.* (*op. cit.*) that the Indian eel might breed in fresh waters and thus behave differently from the European eels.

(b) *Scales as indicative of age and growth of the adult eel*

Structure of scales.—Scales of *Anguilla bengalensis* differ markedly in shape and texture from those of other teleostean fishes, being flat and elongated-oval in shape. However, abnormal scales assuming a variety of shapes (*viz.* circular, dumb-bell and L and T shaped) are not uncommon. Scales lie embedded in the skin and 'are placed in individual sacs in the dermal tissue, with no connections with the epidermal covering' (Waly, 1940). The arrangement of scales in the skin varies slightly, but generally the first row of scales immediately above and below the lateral line lie obliquely at an angle of about 45° to it, directed either forwards or backwards. In the subsequent rows above and below these, the scales lie, generally, at an angle of 90° to those in the preceding rows, the general effect being 'somewhat like parquet flooring' (Frost, 1945) (*vide* Plate XXV, fig. e).

Waly (*op. cit.*), after a detailed study of the scales of *Anguilla vulgaris*, has concluded that the scales comprise of concentric rows of 'oval, round or polygonal loculi, formed of closely applied fibres, with inter-communicating delicate threads, coursing from the external to the internal surface of the scales. These loculi are separated by their common walls. In bigger specimens the rows of loculi are arranged in concentric zones separated by narrow concentric bands of fibrous tissue'. In the scales of *A. bengalensis* also such a zonation is clearly discernible (*vide* Plate XXV, figs. a, b and c). The number of zones in a scale increases generally in direct proportion to the size of the fish. The bands of fibrous tissue or growth rings lie more or less parallel to the margin of the scale and may either be complete, extending all round or incomplete appearing only as hoods or caps on the longitudinal axes of the scale.

Size and age at which scales are formed.—Divergent views have been expressed by various workers regarding the size and age at which scales first appear in eels. Gemzoe (1908) has concluded that scales appear two years after the metamorphosis of the leptocephalus, while Ehrenbaum and Marukawa (1913) have shown, with respect to the European species, that size rather than age determines the appearance of scales. They and some others are of the opinion that scales begin to form between the second and sixth (usually fourth) year of the life of the eel in fresh water. Petersen (1894) observed that, as a general rule, scales are completely formed in

eels when they are about 18 cm. in length. Waly (*op. cit.*) could not detect scales in *A. vulgaris* of 11 to 14 cm., but found them in specimens over 18 cm. in length. Frost (1945) stated that in *A. anguilla* scales are formed during the eel's 2nd to 6th year of life in fresh waters.* All the workers are of the view that scales first appear on either side of the lateral line in the anal region.

In the present investigation, entire skins of elvers, ranging between 80 to 200 mm. in length, were examined to ascertain the size at which scales form. Skins of elvers could easily be peeled off from the underlying muscles after making an incision initially with a sharp scalpel. An entire skin from each specimen examined was stained in 1% solution of alizarine red, after initial treatment with 2% solution of potassium hydroxide. The stained skin was cleared and examined in glycerine. Another specimen of skin from each of the specimens of the same length was first cleaned of the muscle and tissue fibres attached to its inner side by scraping gently with a sharp scalpel and was then immersed in petrol for about 12 hours to dissolve adhering fat. Later the skin was bleached in a mixture of hydrogen peroxide, ammonia and distilled water, and was cleared and examined in glycerine.

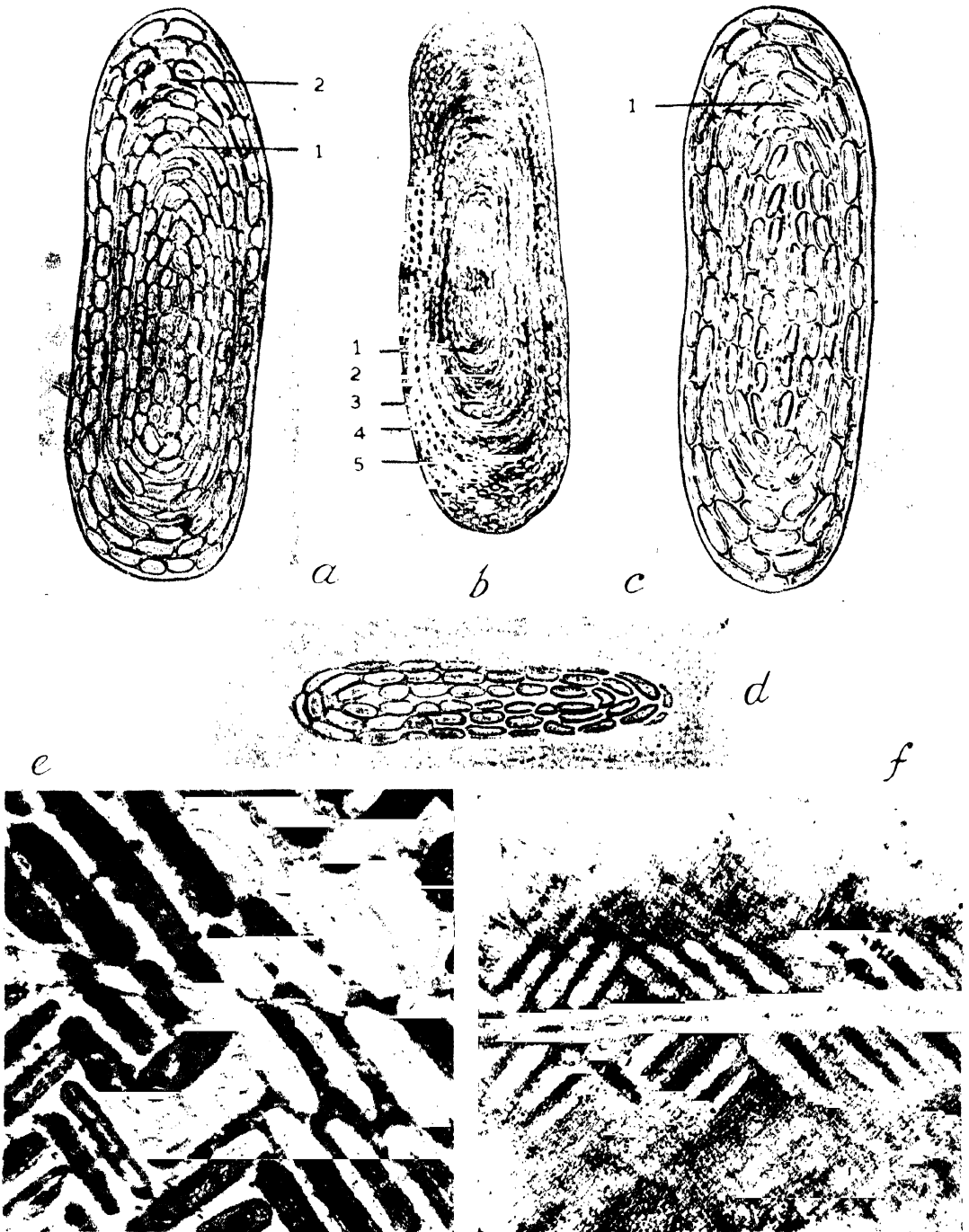
A microscopic examination of the skins so prepared revealed that in eels less than 110 mm. in length there was no indication of scale formation. In bleached skins of eels 110 to 114 mm. long, oval areas having the general shape of scales were clearly discernible, on either side of the lateral line in the caudal region, at a distance of about $\frac{1}{5}$ the body length from the tip of the tail. In eels 110 to 111 mm. long these oval areas are usually devoid of any loculi or platelets (Plate XXV, fig. f), whereas in specimens 112 to 114 mm. in length, loculi can be observed to be developing on these oval areas. These areas are obviously the basic fibrous tissues of the scales in which loculi are formed due to the deposition of particles of calcium salts. Fully-formed scales could be seen in some specimens 112 mm. long. At this stage only one row of scales is present on either side of the lateral line in the caudal region, commencing at a point 25.2 mm. in advance of the tip of the caudal fin and extending up to a distance of another 13.5 mm. anteriorly.

The size of the earliest scales (Plate XXV, fig. d) ranges from 0.240 to 0.336 mm. As the eel grows in length, the scale area extends simultaneously in all directions. Thus the number of rows of scales dorsal and ventral to the lateral line increases with the increase in length of the elver. An elver 122 mm. long has, generally, three rows of scales covering the area between 35 mm. and 11.89 mm. anterior to the tip of the caudal fin. A 205 mm. elver has usually scales covering its entire body. The appearance and extension of scales is diagrammatically represented in Text-fig. 6.

From the above observations, it is evident that the view hitherto held, that in eels of the genus *Anguilla* scales first appear in the anal region, does not hold good in the case of *A. bengalensis*. Though fully formed scales could be made out in some specimens 112 mm. long, in certain cases no scales were found even in specimens 115 mm. long. But all the specimens examined over 116 mm. in length had well-defined scales in the caudal region. Hence it may be concluded that in *A. bengalensis* the scales appear for the first time on the body when it is 112 to 116 mm. in length. Elvers of this length must have spent only six to seven months in fresh waters after metamorphosis (*vide* page 267). These findings are considerably different from those made on European eels.

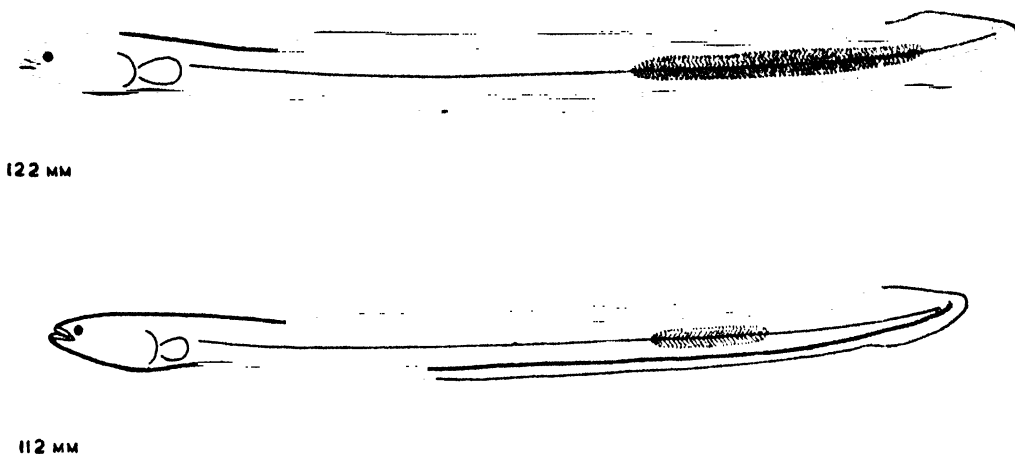
Rahimullah *et al.* (*op. cit.*) could not detect any scales in skin scrapings of *A. bengalensis* 6 inches (about 152 mm.) long collected from Nizamsagar. They could find scales without annual rings only in specimens 11 inches (about 280 mm.) long. In specimens about 152 mm. long collected from the River Hooghly scales were fully formed in the caudal region nearly up to the vent. As the authors have not

* The age at which scales form was deduced by most of the workers cited above from the difference in readings between otoliths and scales.



Scales of *Anguilla bengalensis*—

- FIG. a. Scale showing two rings.
 „ b. Scale showing five rings.
 „ c. Scale showing one ring.
 „ d. Photomicrograph of a skin preparation of a 114 mm. long elver showing a fully formed earliest scale *in situ*.
 „ e. Photomicrograph of a skin preparation of a 380 mm. long eel showing the arrangement of scales *in situ*.
 „ f. Photomicrograph of a skin preparation of a 111 mm. long elver showing the earliest scales (oval areus) prior to the formation of loculi.



TEXT-FIG. 6. Appearance and extension of scales in the elvers of *A. bengalensis*.

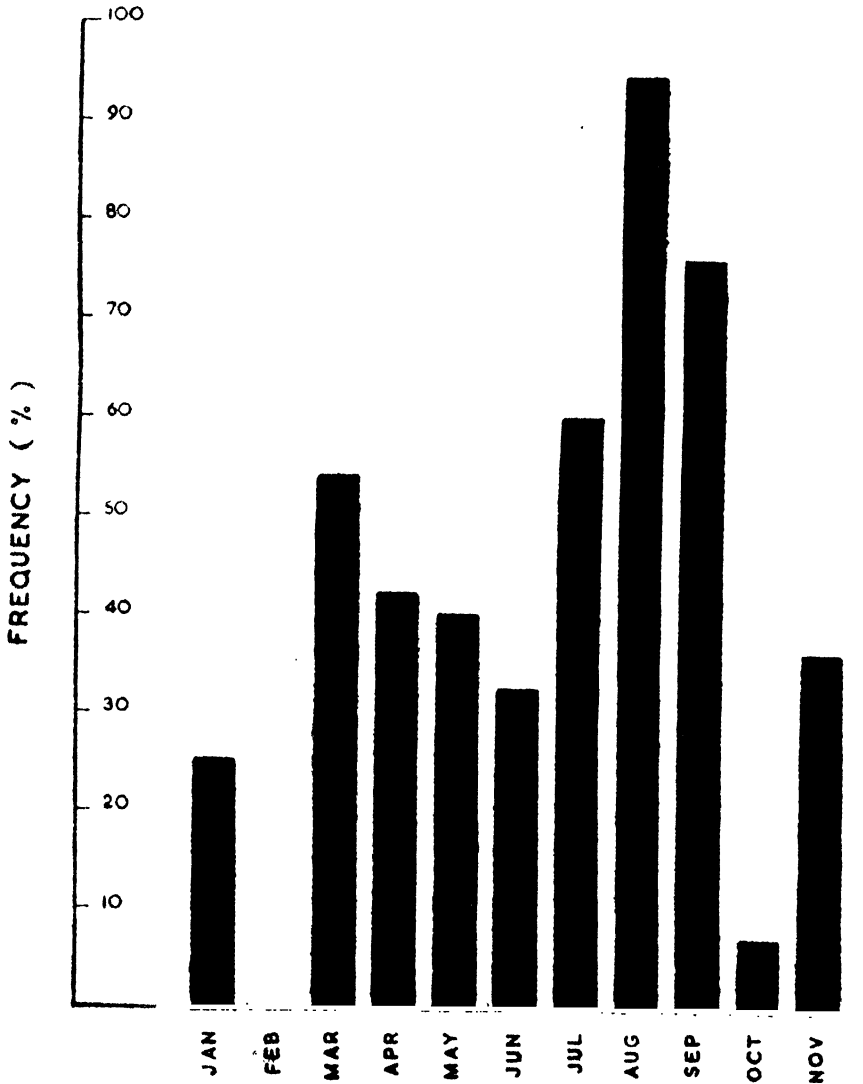
mentioned the part of body from where skin scrapings have been examined it would appear probable that their failure to detect scales in 6 inches long specimens was because they had not examined the skin in the region posterior to the vent.

Growth of scales and appearance of growth rings.—Gemzoe (*op. cit.*) has established that the zones on the scales of the European eel are annual in character. Observing the growth of the scale, he concluded that the larger loculi of the zone were laid during the period of active growth, and that they became gradually smaller and smaller towards the edge, till finally they ceased to be laid during the period of little or no growth. The absence of loculi at the edge of the zone caused the rest of the zone to be framed, as it were, in a ring of fibrous tissue. Gemzoe (*op. cit.*) and Ehrenbaum and Marukawa (1913) observed that growth of scales went on from the end of June to the end of August. Frost (1945) found scales showing new growths at their edges in Windermere eels, during July, August and September, and concluded that the scale growth ended by October. She, however, found some difficulty in determining the growing edge and stated that information on that point was somewhat inconclusive.

During the present investigation, new growing edges were observed generally all round the scale as transparent margins, being broader along the long axes and comparatively narrow along the transverse edges, thus conforming to the general shape of the scale. Sometimes these new growing edges appeared only as broad caps on the long axes of the scale. After the formation of these transparent edges, loculi began to appear on these, first faintly and later becoming more and more distinct. The appearance of loculi did not take place on the entire new growing edge, but a narrow band of uncalcified fibrous tissue is left vacant, framing, as it were, the preceding zone of loculi. Hence in *A. bengalensis* the new ring is laid at the time of resumption of scale growth.

Scales showing such new growing edges were found during all the months of the year, though their frequency varied from month to month. Further, the new growing edges did not appear on all the scales of the same fish at the same time. The maximum and minimum percentage of eels having scales with new growing edges (irrespective of the percentage of scales of individual specimens showing such growths) during various months of the year is 94.4 and 7.1 respectively (Text-fig. 7). During July, August and September the percentage of eels having scales with new growths, as well as the percentage of scales in individual eels bearing new growing edges, is significantly higher than during other months. The results of observations on the nature of scale margins during various months of the year are

delineated in Text-fig. 7, from which it is clear that there is no complete cessation of scale growth in any part of the year. But July, August and September appear to constitute the period of active growth, whereas October to February appear to form a period of retarded growth. As new rings are laid at the time of resumption of scale growth, it is concluded that, in a majority of cases, new rings are formed in the months of July, August and September. The data show that this is by no means the only period of the year when rings are laid, as formation of new growing edges and consequently of growth rings, though in a smaller percentage of eels, is observed during other months of the year also. Hence the utilization of scales for determining age must necessarily involve a certain degree of error unless large samples are examined.



TEXT-FIG. 7. Histogram showing the percentage of eels, exhibiting new growing edges on scale margins, during different months of the year.

The utilization of scales for the estimation of age in the eel is somewhat complicated by the peculiar nature of the scales. A wide variation exists in the size of

the scales taken from the same as well as different parts of the body. Further, scales taken from different areas of the body of the same eel do not always show the same number of rings and such a difference exists in a smaller degree even in scales taken from any one region of the body. The number of rings present on a scale is not necessarily in direct proportion to the size of the scale, a smaller scale sometimes recording more annual zones than a larger one taken from the same region of the body. In view of this, it is not possible to resort to the method of back calculation in the scale studies of the eel. A similar conclusion was arrived at by Frost (*op. cit.*).

In the light of the above observations, scales were used in the present investigation for the estimation of age only, and not growth. The high measure of agreement observed between the estimates of age, by length frequency analysis and scale study, shows that the rings on the scales can be utilized with some degree of caution for age determination of the species.

Scales from nearly 350 specimens ranging from 125 to 666 mm. were studied. They were removed for examination, from an area 20 mm. long (on either side of the lateral line), the mid point of which is about 1/5th of the body length, anterior to the tip of the tail. Selection of scales for study was made from this region, in preference to the usual practice of taking scales from the anal region, because in *A. bengalensis* scales first appear in this area and hence are the oldest. 25 normal scales were selected and cleaned in a weak solution of potassium hydroxide, and mounted for microscopic examination. The growth rings could be clearly seen even in unstained scales. The maximum number of rings found on any scale in the sample was recorded separately for each fish. The usual practice of considering the maximum number of rings found on the oldest scales, as indicative of the correct age of the fish (*vide* Gemzoe, *op. cit.*, Frost, *op. cit.*), was followed in the present investigation. It was ascertained by taking samples of scales from different regions of the body, that the maximum number of rings found in the sample of 25 scales taken from the caudal region, as described, represented the true maximum for any particular individual. Marcus (1919) has concluded that, in certain years, zones of loculi are not laid down on scales of slowly growing eels and that partially formed zones or caps are manifestations of this possibility. Therefore, following his procedure, even partially developed zones of loculi, appearing sometimes only as caps on the long axes of the scale, were considered as representing annual growth.

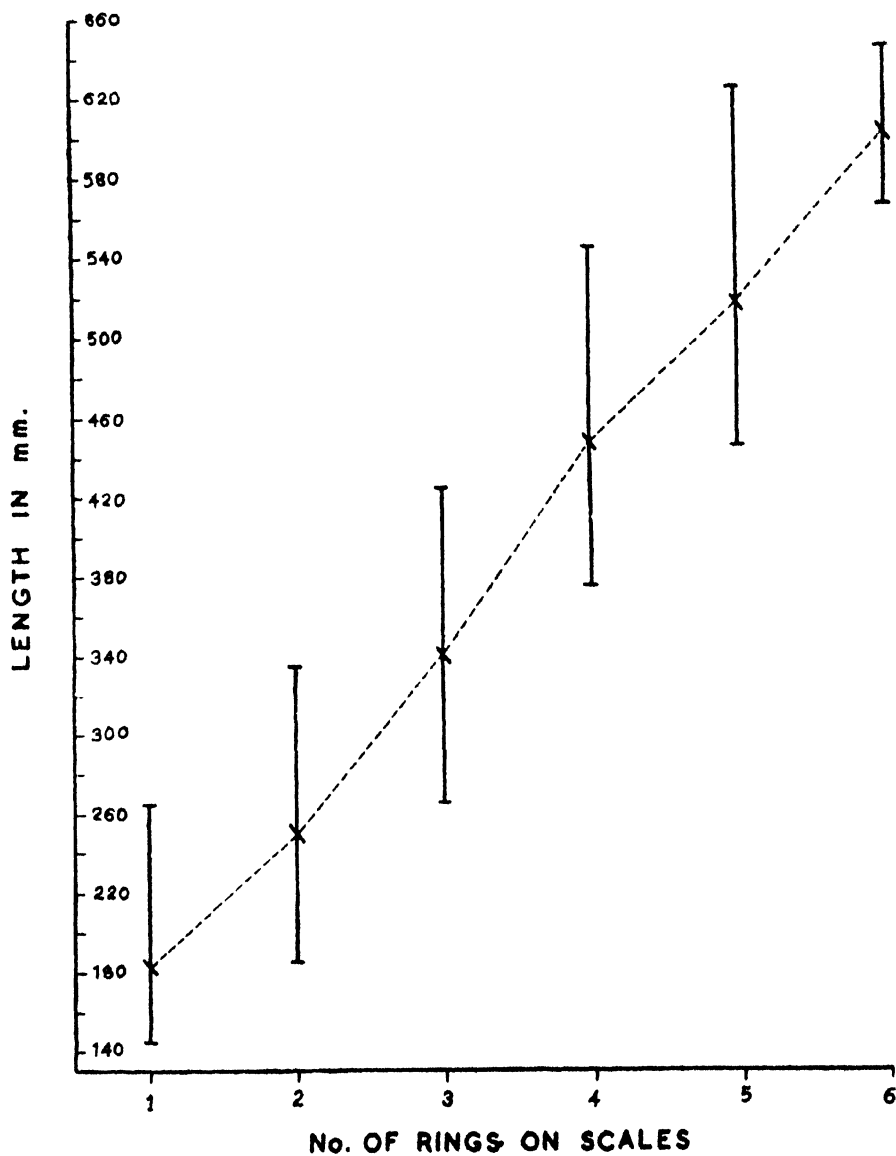
Eels having the same number of maximum rings on their scales are taken, in this investigation, to represent a single age class. The results of scale readings are given in Table V and Text-fig. 8. The average lengths of eels having a maximum number of one to six rings are given in Table VI, along with the age classes as determined by length frequencies.

It was pointed out earlier that immigration of glass eels (after metamorphosis) into estuaries commences late in October every year and continues up to March next year, though the maximum influx takes place in the months of January and February. Scales begin to form about six months after the date of immigration, and the first ring is laid in a majority of individuals during the months of July, August and September of the succeeding year (i.e. second year after immigration into fresh waters). So in the earliest immigrants (*viz.* those that enter the estuaries in the month of October) the first ring is laid in a majority of individuals after about a year and nine months from the date of immigration, whereas in the latest immigrants (*viz.* those that enter the estuaries in March) the first ring is laid after approximately a year and four months. Hence the first ring on the scales of *A. bengalensis* may be considered to have been laid when the eels attained an age ranging between 1 year 4 months and 1 year 9 months, but since the maximum influx of elvers takes place in the months of January and February, it may be said that in a large percentage of eels, the first ring is laid after about $1\frac{1}{2}$ years from the date of their entering the estuaries.

TABLE V

Length range of eels having one to six rings on scales

Range of T.L. in mm.	Number of specimens having rings on scales						Total
	I	II	III	IV	V	VI	
140-149	10	
150-159	11	
160-169	20	
170-179	22	
180-189	11	1	
190-199	13	4	
200-209	8	5	
210-219	11	3	
220-229	4	8	
230-239	1	10	
240-249	3	9	
250-259	1	9	
260-269	2	8	1	
270-279	..	5	5	
280-289	..	4	3	
290-299	..	3	4	
300-309	..	4	2	
310-319	..	1	4	
320-329	..	1	11	
330-339	..	1	6	
340-349	5	
350-359	5	
360-369	5	
370-379	5	2	
380-389	9	3	
390-399	1	1	
400-409	2	1	
410-419	1	3	
420-429	1	6	
430-439	2	
440-449	4	2	..	
450-459	1	4	..	
460-469	4	
470-479	3	1	..	
480-489	1	
490-499	1	
500-509	1	4	..	
510-519	2	
520-529	1	2	..	
530-539	1	
540-549	1	
550-559	2	..	
560-569	1	
570-579	1	..	
580-589	1	..	
590-599	1	..	
600-609	1	..	
610-619	
620-629	1	..	
630-639	
640-649	1	
650-659	
660-669	
670-679	
680-689	
690-699	
Total ..	117	76	70	38	20	2	324
Mean ..	183.56	249.50	340.071	447.132	516.65	602.0	



TEXT-FIG. 8. Range and mean lengths of eels having I to VI rings on scales (range is indicated by horizontal lines and mean by oblique lines).

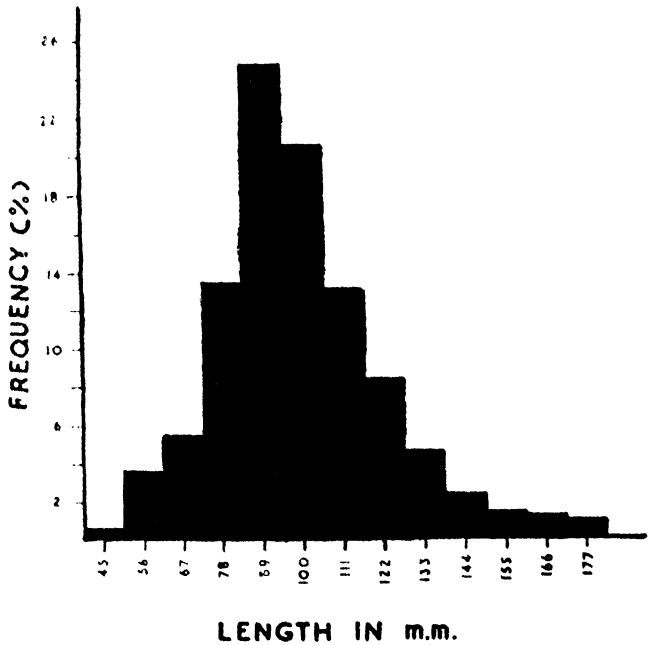
TABLE VI

Mean length of eels in relation to maximum number of growth rings on scales, and class values of modal groups in length frequencies

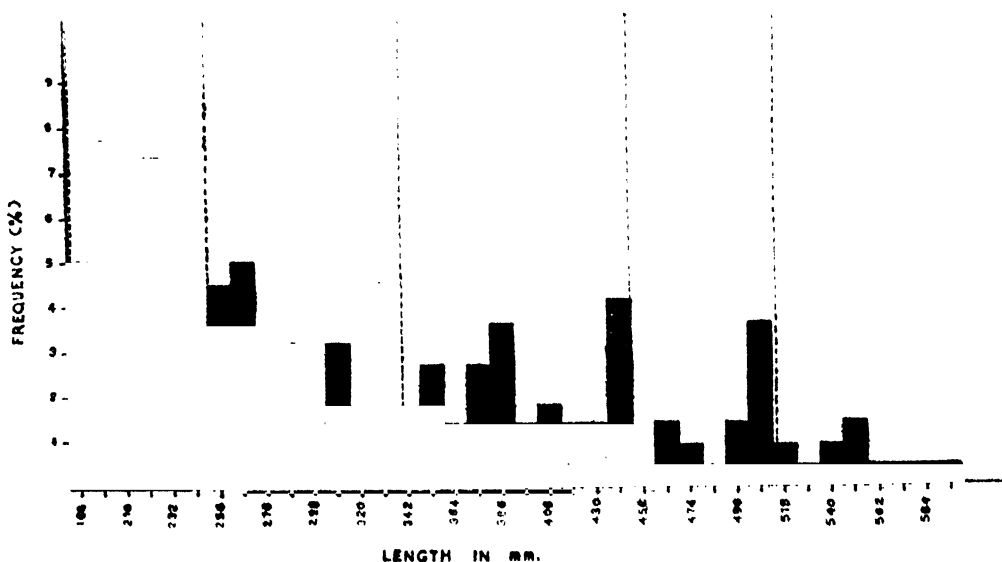
Number of growth rings on scales	Number of eels in sample	Mean length	Length frequencies	
			Age class	Class value of modal group
1	117	183.56	I	210
2	76	249.50	II	265
3	70	340.071	III	331
4	38	447.132	IV	441
5	20	516.65	V	507
6	2	602.00

(c) *Length frequency*

Two length frequency histograms (one for eels measuring 40–183 mm. and another for those more than 183 mm.) for the entire collection of eels made during the year 1952 are given in Text-figs. 9 and 10.



TEXT-FIG. 9. Length frequency histograms of the elvers (less than 183 mm. in length) collected during the year 1952.



TEXT-FIG. 10. Length frequency histograms of eels (ranging between 183 mm. and 600 mm.) collected during the year 1952.

The mean lengths attained by the fish during the first five years of its life, as calculated from the scales, are shown as vertical dotted lines in the histogram. In Text-fig. 9 one distinct mode is noticeable at 80.0 mm. for the O group fish. In Text-fig. 10, five clear modes are discernible, each at 210, 265, 331, 441 and 507 mm., representing I to V year group fish respectively. The small mode at 386 mm. could probably be ignored, as being due to inadequate samples, considering the almost equal distribution of frequencies preceding the main 331 mm. modal group; further the size group represented by this modal class falls within the range of individuals having three rings on their scales. There is another small mode at 551 mm. which could also be ignored since the number of specimens in the sample representing this size group is very small. There appears to be fair agreement between the mean lengths of eels having 1 to 5 rings on scales and I to V modal classes of the length frequency histograms.

SUMMARY

Some aspects of the biology of the Indian fresh-water eel *Anguilla bengalensis* have been studied, based on the collections of eels made from the Hooghly estuary.

The length-weight relationship for eels ranging between 50 mm. and 300 mm. in length is estimated. The condition factor K appears, generally, to increase with the increase in length of the fish. K values for the eels, collected during the later part of the year, appear to be higher than those for eels collected during earlier months.

A study of the food habits of the eel reveals that it is generally omnivorous, though there appears to be a particular preference for fish, which predominate as a single item of diet. The relative predominance of crabs, prawns, insects, megalopa larvae, macro- and microphytes, annelids and miscellaneous items among the stomach contents of the eel is found to be in the order mentioned here.

The immigration of 'glass eels' (newly metamorphosed leptocephali) into estuaries has been found to take place in the colder months of the year, commencing from October and continuing up to March, the peak period of immigration being January and February. The glass eels range between 40 mm. and 58 mm. in length, with a mean length of 50.9 mm. The elvers have been found to attain a length of about 151 mm. registering a growth of 98.0 mm. by the end of the first year of their life in inland waters. The increment in length from month to month varies between 9.0 mm. and 12.5 mm. as revealed by the length frequency studies.

The structure of scales and the age and size at which they first appear is discussed. In *A. bengalensis* it has been observed that scales first appear in the caudal region on either side of

the lateral line of elvers 112 to 116 mm. long and about six months old. The earliest scales measure 0.240 to 0.336 mm. Simultaneously with the growth of the eel the scale area is found to extend until, finally, in an elver 205 mm. long, scales are fully formed on the whole body. These findings differ considerably from those made on European eels.

Observations on the growth of scales and formation of growth rings reveal that the new rings are laid at the time of resumption of scale growth. Though growing edges are found on scales during all the months of the year, the percentage of scales on individual eels bearing new growing edges is significantly higher during July, August and September than during other months. Hence it is concluded that these months constitute the period of active scale growth, and that in a vast majority of eels rings are formed during this season. The somewhat complicated nature of the problem of utilization of scales in the age studies of the eel is discussed, and the conclusion has been arrived that the scales of the eel could be utilized for the estimation of age (not growth) of the fish, with a certain degree of caution, provided large samples are examined. The fairly high measure of agreement observed between the estimates of age, by length frequency analysis and scale study, has also helped to substantiate the view expressed here.

ACKNOWLEDGEMENTS

The author is grateful to Dr. H. Srinivasa Rao, F.N.I., for the interest evinced by him in this investigation and to Dr. B. S. Bhimachar for helpful suggestions. He is thankful to Dr. S. Jones for suggesting this problem and to Dr. T. V. R. Pillay for helpful criticism and suggestions for the improvement of the manuscript. He is also grateful to Shri K. H. Sujansinghani for the help rendered by him in the statistical analysis of some data.

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APPENDIX I

List of organisms found among the stomach contents of A. bengalensis

Annelids

Fresh-water oligochaetes
Earthworms
Polychaetes

Crustaceans

Amphipods
Isopods
Varuna litterata
Megalopa larvae of *varuna* sp.
Paratelphusa sp.
Alpheus sp.
Metapenaeus sp.
Metapenaeus lysianassa
Metapenaeus brevicornis
Palaemon spp.
Caridina sp.

Insects

Gryllotalpa
De-elated termites
Coleopteran larvae
Trichopteran larvae
Mosquito larvae
Beetles
Ants

Fish

Elvers of eels
Gobioid fishes (various species)
Odontambliopus rubicundus
Gobiopterus chuno
Glossogobius giuris
Eleotris fusca
Clupeid fishes (various species). Larvae and adults
Hilsa ilisha
Gadusia chapra
Setipinna phasa
Pama pama
Tetradon spp.
Badis badis
Ambassis ranga
Barbus stigma
Fish eggs

Macro- and microphytes

Aquatic weeds
Blades of grass
Roots and seeds of plants
Spirogyra
Tribonema sp.

Miscellaneous

Shells of gastropod molluscs
Pebbles
Centipedes
Spiders
Sand particles
Debris

Issued September 20, 1957.

BEHAVIOUR OF THE ROCK BEES, *APIS DORSATA* FABR., DURING A PARTIAL SOLAR ECLIPSE IN INDIA

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CONTENTS

	Page
I. Introduction	281
II. Observations	
(a) On 20th June, 1955 (during solar eclipse)	282
(b) On 22nd June, 1955 (normal day)	284
(c) Conclusions	285
III. Summary	285
IV. References	285
V. Addendum	285

I. INTRODUCTION

Little information is available on the behaviour of insects during a solar eclipse. I have been able to find only two relevant references in the literature, viz. that of Newport (1837) on the honey bee in England and of Hukusima (1949) on a Chrysomelid beetle in Japan. Uvarov (1931, p. 103) in his comprehensive review on 'Insects and Climate' refers merely to Newport.

Newport (1837, pp. 306-307) observed that during an almost total solar eclipse in England on the 15th May, 1836, honey bees, *Apis mellifica* Linn., started flocking back to the hives at 2-15 p.m. when the eclipse began and the sunlight was sensibly diminished, and only few were leaving. At 3-15 p.m., when little light remained and the temperature dropped from 20°C. to 15°C., the hives were quiet as in the evenings and not a bee went abroad; also, cocks were crowing. At 4 p.m. when the eclipse was nearly over, full activity was resumed by the bees and they were going abroad in large numbers. He concluded (p. 306) as follows:—'. . . that in proportion to the diminution of light the hives became quiet, and the temperature of the hives decreased until after the eclipse had passed its maximum, when as the light began again to increase, the activity of the hives became restored, and with it a considerable increase of heat'.

The paper of Hukusima (1949) on the Chrysomelid beetle, *Phyllotreta vittata* Fabr., has not been accessible to me.

During the partial solar eclipse which occurred in India on the 20th June, 1955, I was able to observe the behaviour of the rock bees, *Apis dorsata* Fabricius, on a hive in New Forest near Dehra Dun (Uttar Pradesh, ca. 600 metres altitude). The hive (Text-fig. 1) whose flat surface measured about 50 × 60 cm. was situated at a height of about 12 metres from the ground, on a drain pipe on the southern face of a high quadrangle wall. The hive was so situated that the sun shone directly upon it in the mornings.

For the effect of solar eclipse on other animals, *vide* Appendix.

The eclipse at Dehra Dun lasted about 1 hr. 43 mins. (from about 07.37 hrs. to about 09.20 hrs.) in the morning, and was partial, the shadow covering about one-fourth of the sun at the maximum (Text-fig. 2).

Observations were taken with the aid of a field binocular from a distance of about 3 metres from the hive. As during the period of observation the bees were continually both leaving and returning to the hive, the total number of such bees was taken as a convenient and easily observable index of *bee activity* (Tables 1 and 2). For comparison, the bee activity was also recorded two days later on a 'normal' day.

I was assisted in making and recording the observations, particularly in making bee counts, by my children, Vimla, Sarla and Ganpat Singh, for whose enthusiasm and help I record my thanks.

II. OBSERVATIONS

The following is a record of the observations carried out first, during the eclipse (20th June) and, secondly, on a normal day (22nd June).

(a) ON 20TH JUNE, 1955 (DURING SOLAR ECLIPSE)

TABLE 1

Activity of bees Apis dorsata Fabr. (total number of bees leaving and returning to the hive per minute) before, during and after the partial solar eclipse on 20th June, 1955, at Dehra Dun (cf. Table 2)

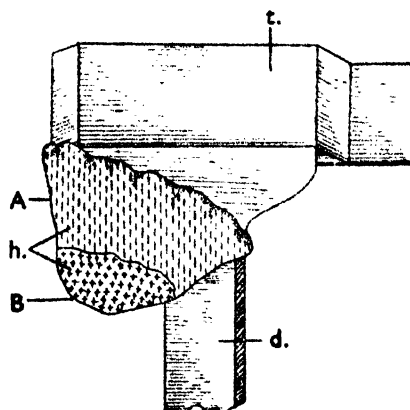
Note.—The individual observations were for 15–30 seconds and the figures so obtained were then calculated for one minute.

Time (hrs.)	Number of bees leaving and returning to hive per minute	Remarks
07.25	20	Cloudy.
07.37	20	Cloudy. Approximate announced time of beginning of eclipse.
08.05	28	Eclipse fairly advanced.
08.30	160 }	Eclipse advanced; about maximum.
08.45	172 }	
09.00	90	Eclipse declining.
09.15	28	Eclipse nearing end.
09.26	34	Eclipse over by now.
09.31	24	

(i) Condition of bees just before the eclipse

07.25 hrs.—The sun is under clouds. The bees are thickly packed on the hive and are generally quiet, but it is possible to divide the hive into two portions in this respect, thus: (i) The upper two-thirds (Text-fig. 1, *A*) in which the bees are very quiet and there is hardly a movement in the mass, except that occasionally a bee here and a bee there shows slight movements; hardly any bee leaves this part of the hive or returns to it. (ii) The lower one-third (Text-fig. 1, *B*) where the bees are distinctly more active. They move their bodies and legs as if for mutual adjustment. Occasionally, a bee leaves the hive or returns to it—this occurs at the rate of about 20 individuals (total of both categories)* per minute, the number of bees leaving and returning being about equal.

* Throughout this paper this figure represents the *total* of both categories, i.e. of those leaving and those returning to the hive.



TEXT-FIG. 1. The hive of the rock bee, *Apis dorsata* Fabr., which was kept under observation during the partial solar eclipse at New Forest, Dehra Dun, on the morning of 20th June, 1955.

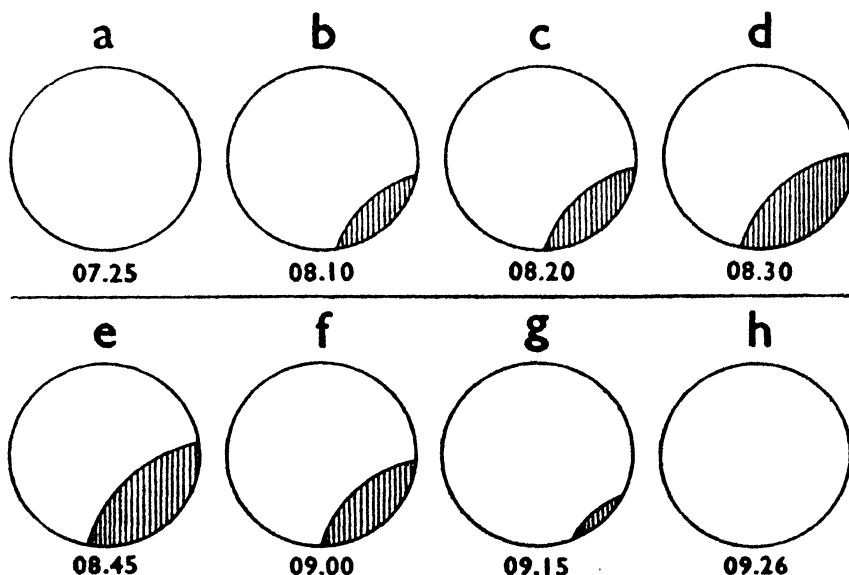
A—upper portion of hive (where the bees were comparatively inactive); B—lower portion of hive (where the bees were comparatively active); d.—drain pipe; h.—bee-hive; t.—drain tank.

(ii) *Condition of bees during the eclipse*

07.37 hrs.—The sun is under clouds. This is the approximate time of the beginning of the eclipse. The bees are behaving as at 07.25 hrs. The number of bees leaving and returning to the hive is about 20 per minute.

07.45 hrs.—It is still cloudy, but some sun rays are partly shining through. The bees are behaving as before.

07.50 hrs.—The sun is under heavy clouds. The bees are behaving as before.



TEXT-FIG. 2. The solar disc as seen at New Forest, Dehra Dun, on the morning (07.25 hrs. to 09.26 hrs.) of 20th June, 1955, during the partial solar eclipse. The shaded portion represents the approximate extent of the sun's disc covered by the moon. The time, in hours, is indicated.

08.05 hrs.—The sun is out of the clouds; the eclipse is fairly advanced. The bees appear to be a little restless in the lower part of the hive. The number of bees leaving and returning to hive is about 28 per minute. In the upper portion of hive the bees are quiet as before.

08.20 hrs.—The sun is visible; the eclipse is advancing. The bees are behaving as at 08.05 hrs.

08.30 hrs.—The sun is alternately in and out of the clouds; the eclipse is nearing the maximum. In the lower part of hive the bees are becoming much more restless than before; the number of bees leaving and returning to hive is about 160 per minute. In the upper part of the hive the bees are as inactive as before.

08.45 hrs.—The sun is alternately in and out of the clouds; the eclipse is almost at the maximum or perhaps a little past it (Text-fig. 2). In the lower part of hive the bees are restless as at 08.30 hrs.; the number of bees leaving and returning to hive is about 172 per minute. There is no change in the activity of bees when the sun goes in and out of the clouds.

09.00 hrs.—The sun is fully out of the clouds; the eclipse is declining. In the lower part of hive the bees are now less restless; the number of bees leaving and returning to hive is about 90 per minute. In the upper part of the hive the bees are comparatively inactive as before.

09.15 hrs.—The sun is now out of the clouds; the eclipse is nearing the end. In the lower part of hive the bees are much less restless than before; the number of bees leaving and returning to the hive is now only about 28 per minute. In the upper part of the hive the bees are inactive as before.

(iii) *Condition of bees just after the eclipse*

09.26 hrs.—The sun is visible; the eclipse is over by now. In the lower part of the hive the bees are behaving as at 09.15 hrs., i.e. they show only mild restlessness; the number of bees leaving and returning to the hive is about 34 per minute. In the upper part of hive, however, the bees, which had all along been very quiet, are now a little restless, and out of several hundred bees, all in the same vertical position and packed closely, a few (5 or 6) have now moved to a slanting position.

09.31 hrs.—The sun is free from clouds. In the lower part of the hive, the bees are behaving as at 09.26 hrs., i.e. are only mildly restless; the number of bees leaving and returning to hive is about 24 per minute. The upper part of the hive shows no change.

(b) ON 22ND JUNE, 1955 (NORMAL DAY)

TABLE 2

Activity of bees, Apis dorsata Fabr. (total number of bees leaving and returning to the hive per minute), on a normal, sunny day (22nd June, 1955) at New Forest, Dehra Dun. (Same hive as in Table 1)

Note.—The individual observations were for 30 seconds and the figures so obtained were calculated for one minute.

Time (hrs.)	Number of bees leaving and returning to hive per minute	Remarks
08.00	50	Sun shining directly on hive.
08.15	50	Ditto.
08.30	56	Ditto.
08.45	62	Sun not shining directly on hive.
09.00	64	Ditto.
09.30	50	Ditto.

For comparison with observations during the eclipse, the behaviour of the bees in the same hive was observed two days later, on the 22nd June, 1955, with the following results. The number of bees leaving and returning to the hive is given in Table 2, the number leaving and returning being about equal.

08.00 hrs.—The sun is *out* and shining directly on the hive; there are no clouds. As on the 20th June, the bees are more active in the lower part of hive than in the upper. The upper portion is distinctly more active than on the 20th June, although still less active than the lower one.

08.15 and 08.30 hrs.—There is no change.

08.45 hrs.—The sun is as before but is not now shining directly on the hive. The bees all over the hive are more active than before.

09.00 hrs.—The condition of bees is generally as at 08.45 hrs.

09.30 hrs.—The condition of bees is generally as before, but the number of bees leaving and returning to the hive is rather fewer.

(c) CONCLUSIONS

It would appear that during the partial solar eclipse on the 20th June, the rock bees became distinctly restless and more active. This was evident both by general visual observation as well as by counting the number of bees leaving and returning to the hive. The total of such individuals (both leaving and returning) rose from 20 per minute at the beginning of the eclipse to 160–172 at the maximum eclipse, and then gradually fell with the decline of the eclipse, the count being about 24–34 at the end. Comparison on a normal sunny day two days later (22nd June) during roughly the same period gave this count as 50–64; no marked rise or fall was noticeable.

III. SUMMARY

1. The behaviour of the rock bees, *Apis dorsata* Fabr., was observed in a hive at New Forest, Dehra Dun (Uttar Pradesh), during a partial (about one-fourth) solar eclipse, on the 20th June, 1955, which lasted for about 1 hr. 43 mins. (from about 07.37 hrs. to 09.20 hrs.). For comparison, the behaviour of the bees in the same hive was observed two days later (on the 22nd June).

2. It was noted that during the eclipse the bees became distinctly restless and more active. The number of bees leaving and returning to the hive rose from about 20 at the beginning of the eclipse to 160–172 at the maximum eclipse, and then gradually fell to about 24–34 with the decline of the eclipse. No such marked rise and fall was noticed on the 22nd June, the figures being 50–64.

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V. ADDENDUM

After this paper was ready for the press, two recent observations on the effect of solar eclipse on animal behaviour came to my notice and deserve mention.

Pillai (1956) made some observations during a partial solar eclipse* at Trivandrum (southern India) on the 14th December, 1955, which commenced at

* Pillai did not mention the extent of the eclipse. On an enquiry from the Officer-in-Charge, The Observatory, Trivandrum, the latter informed me (in his letter dated 25th March, 1957) that the solar eclipse observed at Trivandrum on the 14th December, 1955, was 'a partial one and the maximum extent of it was about 80%'; and further that the 'total time of duration was 4 hrs. 21 mins.'; and 'it was maximum at 12.55 p.m.'.

10.37 a.m. and lasted 4 hrs. 21 mins. At 12.35 p.m. it was so dark that lights had to be put on inside the rooms. The temperature fell from 82.6°F. (ca. 28.1°C.) at 12.00 hrs. to 80.6°F. (ca. 27°C.) at 13.00 hrs. and 80.4°F. (ca. 26.8°C.) at 14.00 hrs.; there was also a slight fall of atmospheric pressure and a rise in humidity by 9 per cent. He observed several birds and mammals in the zoological garden during the eclipse and concluded that 'contrary to popular notion, animals either captive or free display little or no responsive behaviour during a solar eclipse'.

Marsden (1956) observed during the solar eclipse in 1936, in Japan, that until the eclipse was total such birds and domestic animals as came to his notice behaved in no way out of the common, but as soon as the eclipse was total (about 1 p.m. local time), it was as dark as in a clear moonlight night in India, and 'all animal noise and movement ceased forthwith'. Fifty-five seconds later when the moon's shadow on the earth passed away equally suddenly, 'the usual animal noises of early dawn were heard on all sides—cocks crowing, etc.—and normal movement was resumed'.

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Dr. S. L. Hora

SUNDER LAL HORA

(1896–1955)

OBITUARY NOTICE

by M. L. ROONWAL, F.N.I., *Director, Zoological Survey of India, Calcutta*

(With a photograph)

On the 5th December, 1955, while presiding over a meeting of the Asiatic Society of Bengal, Calcutta, and in which he read a paper on the significance of *mahi-o-maratib*, a fish-insignia of the Moghul times, Dr. S. L. Hora was seized with an attack of coronary thrombosis, his second in the last three years, and passed away peacefully on the 8th December at the age of sixty.

Hora's interest in zoology was kindled rather late; in his early college years he did poorly in the subject. This was a source of much disappointment to him and at the same time spurred him on to study harder. The results of this concentration were soon evident in the high marks he obtained in his examinations. He was awarded a scholarship to continue his honours course in zoology which he duly completed and followed up with completing the Master of Science course at the Government College, Lahore, under Professor J. Stephenson. For this course he took up 'Fishes of Lahore' as the subject of his thesis and started in earnest the collection and identification of the fishes. Although never published, this was Hora's first paper on systematic ichthyology, a subject on which he, in due course, became an authority. Dr. N. Annandale, the then Director of the Zoological Survey of India, who visited Lahore about that time was greatly impressed with Hora's collection and display of fishes in the museum and invited him to help him (Annandale) in his forthcoming Seistan tour.

Hora's father, finding his son drifting into science instead of business, warned him that his subject, zoology, would never earn him a decent livelihood. Hora accepted his father's advice and started business at Simla, and would have become a full-fledged businessman but for Annandale's call to Calcutta. Professor Stephenson exercised his influence, and Hora agreed to join Dr. Annandale. This was a turning point in his career, and had Professor Stephenson and Dr. Bains Prashad not encouraged him to go to Calcutta, Indian ichthyology would perhaps never have heard of Hora. There is no doubt that among the early influences, which moulded Hora's career in zoology, that of Dr. Annandale of the Zoological Survey of India, which Hora joined as a Research Scholar in 1919, was the most dominant and decisive. In all his writings he endeavoured to follow the master's methods, and, to the end of his days, he never tired of speaking of Annandale with deep reverence and affection.

As a zoologist Hora remained throughout his life devoted to fish as a subject of his studies. But his interests were catholic and his sweep extended to a variety of fields, such as taxonomy, zoogeography, evolution, ecology and history of science, always with fish as the *motif*. But Hora was first and foremost a fish taxonomist and his most outstanding contributions lie in that field. His first paper, jointly with Annandale, was published in 1920 and dealt with the fish fauna of Seistan. From then onward, a steady stream of papers on fish taxonomy emanated from him, covering not only all parts of India but also practically all the neighbouring countries such as Afghanistan, Burma, Malaya, Thailand, China, Indonesia and Iraq. Hora

confined himself almost wholly to the freshwater fishes which comprise about 22 per cent of the Indian fauna, and in this field he was an unrivalled authority. No student of the freshwater fishes of the Oriental Region can afford to ignore the solid and extensive contribution of Hora in that field. He discovered dozens of new species, several new genera and revised numerous families of fishes. For his work he had the advantage of having two excellent Assistants—first, Mr. D. D. Mukerji who died prematurely and then Dr. K. S. Misra who, on Hora's promotion to the Directorship of the Zoological Survey of India, succeeded him as the Officer-in-Charge of the Fish Section of the Survey.

Hora was attracted greatly to theories of organic evolution and contributed excellent material towards them, especially on adaptations in animals inhabiting torrential streams of India with particular reference to the organs of attachment. However, while his field observations were unexceptionable, his interpretations were open to serious dispute. Somehow or other, he seems to have completely ignored the entire gamut of recent researches in the field of genetics which have so deeply influenced our ideas of evolution. To the end of his days Hora continued to believe ardently in the Lamarckian theories—theories which are more or less fully discredited today. It is difficult to explain this curious isolation of Hora from modern developments in ideas on evolution. Possibly, he was too busy to devote much time to reading new material and assimilating new ideas on the subject. I remember vividly how a few years ago, at a symposium on Organic Evolution organized by the National Institute of Sciences of India in Delhi, Hora presented his Lamarckian views and was immediately challenged by a young and knowledgeable geneticist. Hora found defence difficult and ultimately extricated himself by contending that Lamarckianism was his belief and he did not care whether others accepted it or not.

In later years Hora felt greatly involved in what he called the 'Satpura Hypothesis' by which he meant that the similarity between the Malayan fauna and the fauna of Peninsular India without an apparent connecting link (a well-known fact of zoogeography known since the last century) was due to migration from eastern regions to the western *via* the Satpura Range of mountains in central India. This hypothesis, which he first put forward in 1937, was mainly based on evidence from freshwater fishes. Later on, he elaborated it with the assistance of other workers to include diverse groups of animals. In 1949 he organized a symposium on this subject under the auspices of the National Institute of Sciences of India. The symposium threw a flood of light on the existence of the discontinuity of faunal distribution, as mentioned above, but could hardly be said to have provided any positive evidence in favour of the Satpura Hypothesis as an explanation of this discontinuity.

Hora also felt greatly attracted towards studies on the history of science in ancient and medieval India especially with regard to fish and fisheries, and he published a whole series of illuminating papers on the subject.

Hora had a kind and sympathetic heart and loved to encourage young students and workers, providing them freely with all the facilities he could and even going out of the way to help them. With his enemies he never compromised. At the same time, he had great adaptability, and it was this characteristic more than anything else which made him so successful a member of innumerable scientific societies in most of which he held high offices. In fact he was deeply attracted both to the internal politics and the day-to-day working of these societies and there is a belief among his friends that this excessive work told upon his health which broke down towards the later years of his life. Had he not had so many irons in the fire at the same time, he would, perhaps, have lived longer and would have made even greater contributions to science than he did. His weakest spot lay perhaps in the realm of administration where he was prone to be wavering and indecisive and hesitant to shoulder responsibilities. He was happiest in the field—collecting animals and making observations in field ecology, and his great example should be an inspiration

to the younger zoologists. Hora was also happy in the surroundings of scientific societies and other such bodies and took a leading part in their meetings.

Hora considered work as pleasure and even his hobby was the writing of scientific papers. He had the gift of turning his stumbling blocks into stepping stones of success. The number of students trained by him are many, and several amongst them occupy positions of responsibility. In all those who came in contact with him, he inspired a love for hard work. As a man he was gentle and lovable, and as a teacher kind and considerate to a fault.

From 1920 to the end of his career he published about 427 papers on various subjects but mainly on fishes, evolution and adaptation; they appeared both in Indian and foreign journals. His contributions cover all aspects of biology including zoology, physiology, systematics, bionomics, ecology, evolution, zoogeography, palaeogeography and history of zoology. As a fishery expert and a leading taxonomist, the Zoological Survey of India became under him a centre for research on fishes and attracted students and research workers from all parts of India.

A fully indexed list of his publications was issued in a booklet in 1951 by his admirers and friends 'in commemoration of the Silver Jubilee of Dr. S. L. Hora's first contribution to Science'. A complete list of his publications, including a few which appeared posthumously, is given below in a chronological order; two or three of his papers are still believed to be in the press.

ACKNOWLEDGEMENTS

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LIFE-HISTORY OF *BARBULA INDICA* BRIDEL.

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Barbula indica Bridel. is the common leafy moss found growing profusely on damp places and brick walls. It is gregarious in habit, and grows in dense masses forming yellowish-green patches usually intermingled with another moss *Hyophila involuta* (Hooker) Jaeger. The latter can, however, be easily distinguished by its wider leaves, shorter axis and absence of peristome in the capsule. Brotherus (1909), in the second edition of Englar's 'Pflanzenfamilien', mentions the presence of three species of *Barbula* in Bengal; they are:

B. gangetica C. Müllar,
B. comosa C. Müllar, and
B. orientalis C. Müllar.

Since then, Brühl (1931) has reported the presence of *B. indica*, *B. gangetica* and *B. comosa* in Bengal. Brühl and Sarker (1933) have described the morphology of *B. indica*.

Paris (1904) in his 'Index Bryologicus' mentions *Tortula orientalis* as synonym of *B. indica*. The genera *Barbula* and *Tortula* belonging to the family Pottiaceae with similarity in some of the essential morphological features has produced confusion with regard to the position of some of the species (Bartram, 1949; Francis, 1950). So far as the present material is concerned the characters of the gametophore and the sporogonium conform to the species *B. indica* Bridel. in all respects.

Considering the extensive work done and in progress on various aspects of the Hepatics in India, the true mosses appear to have been somewhat neglected though they are not of infrequent occurrence. Very few attempts have so far been made to study in detail the life-cycle of different species of the true mosses. This is essential from the systematic and other points of view. This paper gives a detailed account of the life-cycle and the chromosome behaviour during the meiotic division of the spore mother cells.

MATERIAL AND METHODS

The material has been collected from plants growing in large patches on damp rock surfaces and brick walls in shady places within the college campus.

For general morphological studies, the material was dissected and observed under a Zeiss Stereoscopic binocular microscope.

Formalin acetic alcohol fixative was found to be suitable for general anatomical studies and chrom-oso-mo-acetic acid fixing fluid (Johansen, 1940) for the study of the development of sex organs, embryos and young sporophytes. The material was always washed in running water before fixation to remove soil particles held between the tufts of leaves. Dehydration was carried out in grades of normal butyl alcohol and embedded in paraffin as usual.

Sections were cut 5μ thick for anatomical studies and 3 to 5μ for the study of the development of sex organs and capsule. Material for the anatomical studies was stained in safranin-light green. Heidenhain's iron alum-haematoxylin was found suitable for developmental studies. Orange G was used as counterstain where necessary.

For the study of meiosis, capsules of suitable size were fixed in acetic alcohol (1:2) for one hour and then transferred to 45% acetic acid before squashing in

aceto-carmin. Peak period of division was found to be between 8-30 a.m. and 10 a.m. Best results were obtained where capsules were treated in 0.002 M. 8-oxyquinoline for twenty minutes at 15°-17°C. before fixation in acetic alcohol.

For spore cultures and regeneration studies the following nutrient media were used:

1. Benecke's nutrient solution.
2. Benecke's nutrient agar substrate 2%.
3. Spore culture media (Johansen, 1940).

The nutrient media were sterilized at 5 lb. pressure for 30 minutes.

Spores of only one capsule were inoculated in each petri dish or flask. No monosporous cultures were made. Hanging drop cultures in Benecke's nutrient solution and tap water were made to study the earlier stages of germination. The cultures were illuminated unilaterally from a south-east facing window. During vigorous growth of protonema on solid media, supply of moisture was found to be necessary for the cultures.

For regeneration studies, leaf and axis of the gametophore, brute bodies, capsule and seta were placed on moist filter paper in sterilized petri dishes and in Benecke's nutrient solution.

OBSERVATIONS

(a) *Morphology of the gametophore*.—Plant mass dense, bright yellowish green, soft with erect gametophores 1 to 1.5 cm. long. Axis usually simple yellowish brown in colour except at the young portion; laxly foliose with smooth-walled rhizoids at the base. Rhizoids usually with oblique transverse walls; brown in colour at the mature portion.

Leaves lanceolate, apex obtuse, margin more or less recurved, minutely sinuate due to the projecting papillate walls of the marginal cells near the apex, costa strong, apparently biconvex, ending in a small conical hyaline point slightly projecting beyond the apex of the leaf blade (Fig. 54). The leaves are borne spirally in definite tristichous arrangement near the apex, but further below the definite arrangement is broken down due to torsion and twisting of the axis during development (Fig. 36). Sex organs are usually borne terminally on the main axis and short lateral branches.

The axis of the gametophore develops from a typically inverted pyramidal apical cell having three cutting faces, which cut off segments at regular sequence (Fig. 55). The segments by further periclinal and vertical divisions give rise to cells which contribute to the central and cortical portion of the axis. The leaf initials differentiate from a superficial cell of the axis. Thus the arrangement of the leaves developing from the segments cut off by the three-sided apical cell is typically tristichous.

The axis in cross-section is nearly terete. It is differentiated into two distinct portions: the central cylinder which is composed of thin-walled, narrow, vertically elongated cells, polygonal in cross-sections; while the cortical cells are much larger and contain chloroplasts in the young stage. In mature axis the cell walls of the cortex become much thicker and yellowish brown in colour. The thickness of the walls gradually diminishes towards the centre (Fig. 58). The epidermal layer is not clearly defined, though the superficial cells are mostly isodiametrical.

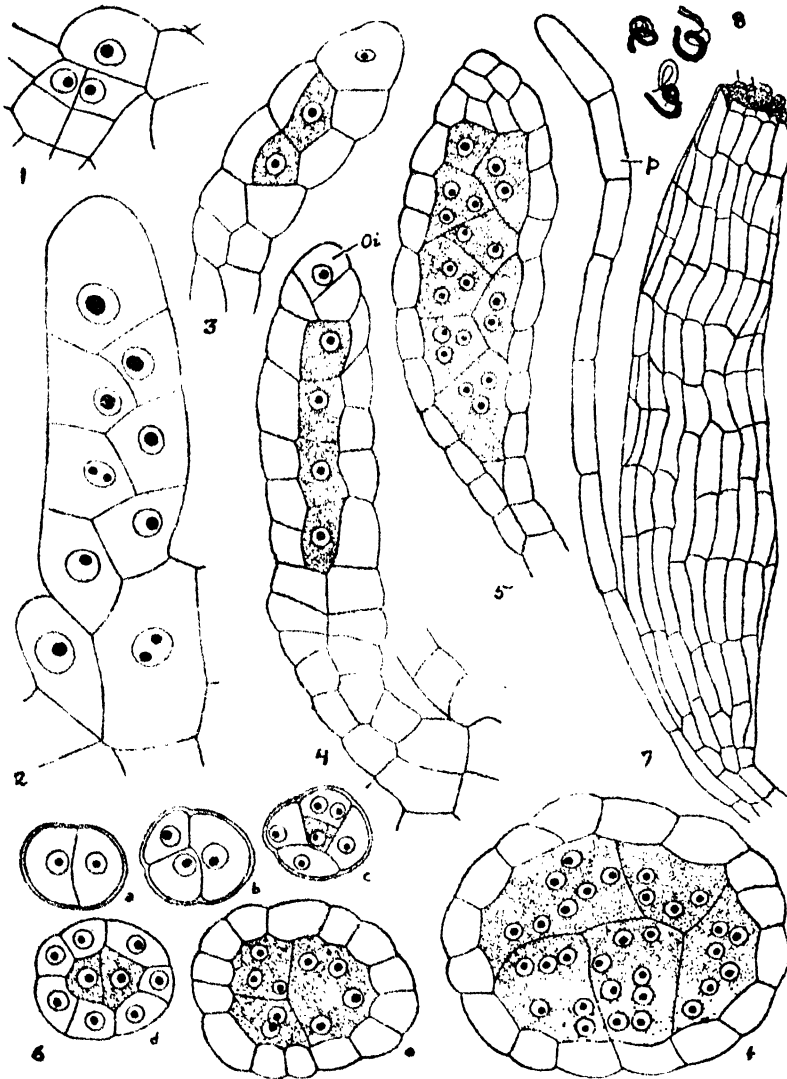
The midrib of the leaf is biconvex in cross-section. Four deuter cells lying in a row in the midrib are surrounded by narrow, thick-walled substeried cells and delimited by large rectangular peripheral cells. The lamina is one cell in thickness; cells more or less isobilateral in cross-section (Fig. 57). The cells of the basal portion of the leaf in surface view are large rectangular, becoming narrower towards the margin and with a fewer chlorophyll grains than those in the apical portion where they are irregularly polygonal, much smaller in size and densely

chlorophyllose. Perichaetal leaves are not distinguishable from the foliage leaves except that the cells of the lamina are uniformly rectangular throughout its length.

Primordia of branches arise from superficial cells of the axis between the bases of two closely imbricated young leaves usually at a short distance below the growing apex (Fig. 56). The cells differentiate as apical cells with three cutting faces; further development is similar to that described in the case of the axis.

(b) *Development of the sex organs.*—The gametophores are strictly dioecious. The plants bearing antheridia and archegonia are not distinguishable and grow in separate or intermingled masses.

The sex organs develop successively from the superficial segments cut off by the apical cell of the axis which forms the narrow head. Long filamentous



FIGS. 1-8. Stages of development of antheridium. Fig. 1. Antheridial initial cell. Figs. 2-5. Different stages of development of antheridium; initial for cover cells = *oi*. Figs. 6a-6f. T.S. showing stages of development of antheridium. Fig. 7. Mature club-shaped antheridium after liberation of the antherozoids; paraphyses = *p*. Fig. 8. Antherozoids. (Original magnifications of Figs. 1, 2 and 6 = $\times 2,000$; Figs. 3, 4 and 7 = $\times 1,500$; Fig. 5 = $\times 1,000$; Fig. 8 = $\times 2,400$.) (Reduced $\frac{1}{2}$ in reproduction.)



FIG. 63



FIG. 64

FIGS. 63-64. Fig. 63. Regeneration of gametophore from mother plant. Fig. 64. Cluster of archegonia with paraphyses. (Original *mags.* of Fig. 63 $\times 20$; Fig. 64 $\times 100$.)

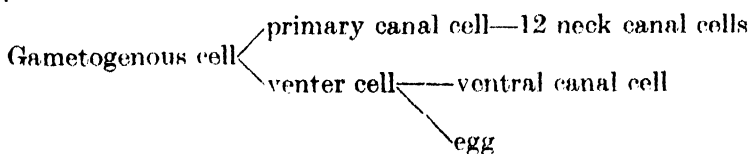
paraphyses, composed of 8 to 10 cells with pointed ends (Figs. 7 and 64), are found intermingled with the antheridia in the male plant, and surrounding the central cluster of archegonia in two or three rows in the female plant. The apical cell which is not directly functional in case of antheridia sometimes continues to develop the axis, so that the antheridial cluster becomes lateral in position. Two to three such lateral clusters were found to occur in some of the mature plants in addition to one terminal cluster.

Early development of the antheridium and archegonium are almost similar. A superficial cell on the head forms a papillate projection with dense cytoplasm and becomes differentiated as an initial cell (Fig. 1). First division of the initial cell takes place in an obliquely transverse plane giving rise to an apical cell with two cutting faces, which cut off segments at regular sequence forming a filament of six to eight segments in case of antheridium and four to six segments in case of archegonium (Figs. 2, 6a, 6b, 9).

In case of antheridium, the apical cell with two cutting faces continues to function. The segments cut off by the apical cell undergo two successive divisions in diagonally vertical plane (the later in a plane at right angles to the former) giving rise to two jacket initials and the central, primary androgonial cell (Fig. 6c). Before the apical cell ceases to function, it cuts off two segments and organizes the cover initials (Fig. 4, oi). The primary androgonial cells divide and redivide in transverse and vertical planes accompanied by the compensating division of the initial jacket cells which form the jacket layer (Figs. 3, 4, 6d). Further development of the androgonial tissue takes place by karyokinesis which is not followed by cytokinesis and wall formation (Figs. 5, 6e, 6f). Subsequently, however, walls are laid down delimiting each nucleus of the androcyte mother cells which lie in blocks corresponding to the wall of the cells of the androgonial tissue. Each androcyte mother cell divides to form two androcytes which lie entangled in a mucilaginous mass within the mature club-shaped antheridium (Fig. 7), the cover at the tip of which can be easily distinguished by the larger size and thicker wall of the cells.

The androcyte cells are metamorphosed into long, slender and coiled bodies with two long cilia attached near one end, forming the antherozoids (Fig. 8). Dehiscence of the antheridium takes place by rupturing of the tip of the antheridium.

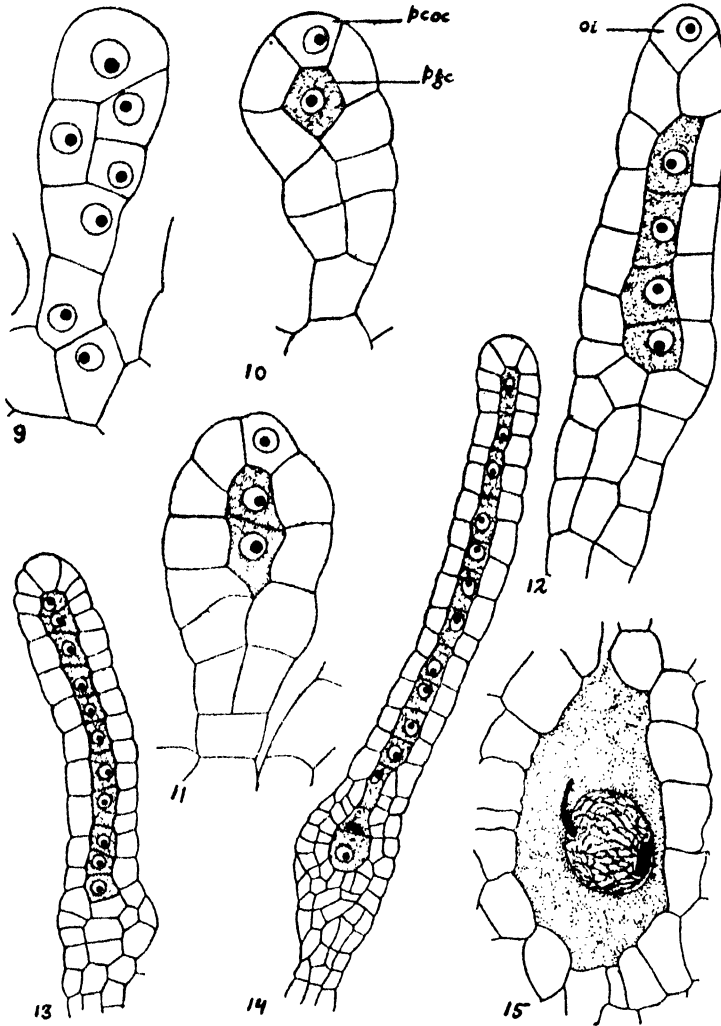
The development of the archegonium begins when the apical cell with two cutting faces after contributing to the stalk portion by an oblique periclinal division develops three cutting faces. The three faces cut off segments which form the peripheral initials. These by vertical divisions give rise to six jacket initials. The apical cell, now functioning as the axial cell, divides successively in a transverse plane. First division results in an upper primary cover cell and lower primary gametogenous cell (Fig. 10). Further development of the gametogenous cell takes place as follows:



Stages of development of archegonium has been shown in Figs. 11 to 14.

Development of the gametogenous cell is accompanied by the compensating divisions of the jacket initials which form the venter and the long neck (Fig. 14). The primary cover cell forms four large cover cells at the tip of the neck.

In mature archegonia the disorganization of the ventral canal cell is followed by the neck canal cells. At the time of fertilization, which is effected in presence of moisture, the cover cells split open the canal of the archegonium. The antherozoids, which swim out of the antheridium in presence of moisture, pass through

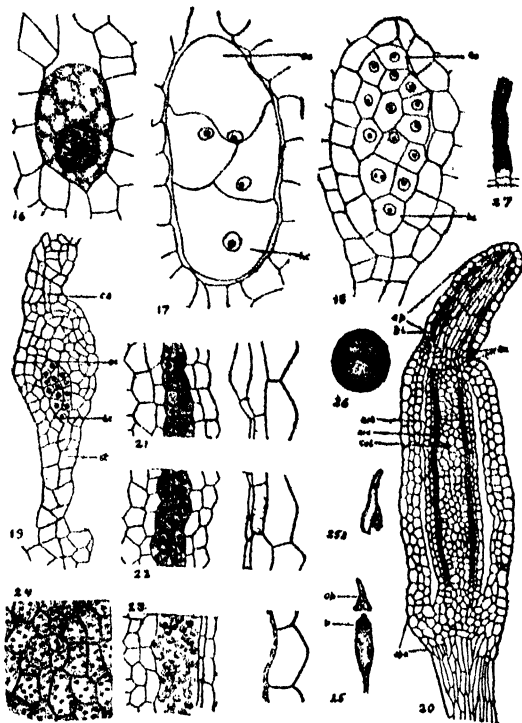


FIGS. 9-15. Figs. 9-14. L.S. showing stages of development of archegonium; primary gametogenous cell = *pgc*; primary cover cell = *pcoc*. Fig. 15. Fusion of the male and the female gametes. (Original *mags.* of Figs. 9-12 and 15 = $\times 2,200$; Figs. 13 and 14 = $\times 700$.) (Reduced $\frac{1}{4}$ in reproduction.)

the neck of the archegonium down to the large, almost spherical egg. On entering the cytoplasm of the egg, the antherozoid loses the two cilia and ultimately fuses with the nucleus (Fig. 15). The cytoplasm of the zygote becomes yellowish brown in colour.

(c) *Development of the sporogonium.*—The development of the sporophyte begins with the enlargement of the zygote (Fig. 16) followed by a transverse division (Fig. 17). Both the epibasal and the hypobasal cells then divide by oblique transverse walls successively, to organize an apical cell with two cutting faces at either end. The apical cells cut off segments at regular sequence forming a long slender embryo (Figs. 18, 19). Development of the embryo at this stage is accompanied by enlargement of the archegonial jacket, the upper portion of which, forming the calyptra, remains perched up on the sporophyte apex till maturity (Fig. 19, *ca*).

The hypobasal apical cell of the embryo, by rapid development, burrows through the stalk of the archegonium and penetrating the apical portion of the



Figs. 16-27. Fig. 16. Zygote. Figs. 17-19. Stages of development of the embryo. Fig. 17. Delimitation of epibasal (*ec*) and hypobasal (*hc*) apical cells. Figs. 18 and 19. Position of the young embryo in the archegonium; calyptra = *ca*; stalk of the archegonium = *st*. Fig. 20. L.S. of the young capsule; operculum = *op*; peristome initial = *pi*; annulus = *an*; archesporium = *arc*; air chamber = *a.ch*; columella = *col*; apophyses = *apo*. Figs. 21-23. Stages of development of the sporogenous tissue. Fig. 24. Surface view of a portion of the apophyses showing stoma. Fig. 25. Mature capsule; peristome = *p*; operculum = *op*. Fig. 25A. Calyptra. Fig. 26. Spore. Fig. 27. A peristome tooth. (Original magn. of Figs. 16 and 17 = $\times 2,200$; Fig. 18 = $1,500$; Figs. 19, 21 to 23 = $\times 150$; Fig. 24 = $\times 250$; Figs. 25 and 25A = $\times 29$; Fig. 26 = $\times 3,000$; Fig. 27 = $\times 1,000$.) (Reduced $\frac{1}{2}$ in reproduction.)

gametophore subsequently gives rise to the long slender seta. Segments cut off by the epibasal apical cell divide periclinally towards the periphery and irregularly towards the centre to form a central mass of tissue surrounded by four layers of cell. The three outermost layers form the amphithecium which is delimited from the inner zone, the endothecium, by formation of an air chamber. At a certain distance below the apex, a transverse row of superficial cells by further anticlinal divisions form a ring of very narrow, thin-walled cells of four to six tiers. These ultimately form the annulus, above which the superficial layer of cells of the sporogonium organize the operculum (Fig. 20). The air chamber delimiting the endothecium and amphithecium extends from near the base of the operculum to the apophyses.

The superficial layer of cells of the endothecium by periclinal divisions give rise to two inner layers, of which the innermost directly functions as the archesporium. The archesporium thus formed is a barrel-shaped tissue, extending up to the rim of the operculum and is one layer in thickness (Fig. 20, *arc*). It surrounds the central column of tissue, the columella, consisting of large cells, which extends through the urn of the capsule into the operculum. The peristome teeth are organized from the single layer of cells corresponding to the outermost layer of the endothecium at the level of the annulus.

The archesporium by division and redivision of its cells forms the sporogenous tissue, six to eight cells thick (Figs. 21, 22), the cells of which later round up and form the spore mother cells. These by reduction division give rise to tetrad of spores (Fig. 23). With the development of the spores, the superficial cells of the columella adjoining the archesporium degenerate to form the spore chamber. The two outermost layers of the endothecium, however, persist up to the time of formation of the spores, thereby separating the air chamber from the spore chamber (Fig. 23). With maturity of the capsule these two layers are pushed towards the amphithecium by enlargement of the spore chamber, and ultimately degenerate. In the meantime the two inner layers of the amphithecium also degenerate, the cells of the outer layer become large and show thickening of the outer tangential wall.

The peristome initials by anticlinal division form long narrow cells, the outer and inner walls of which become thick, the radial walls remaining thin. With maturity the cell contents disappear and the tissue splits along the radial wall to form the peristome teeth.

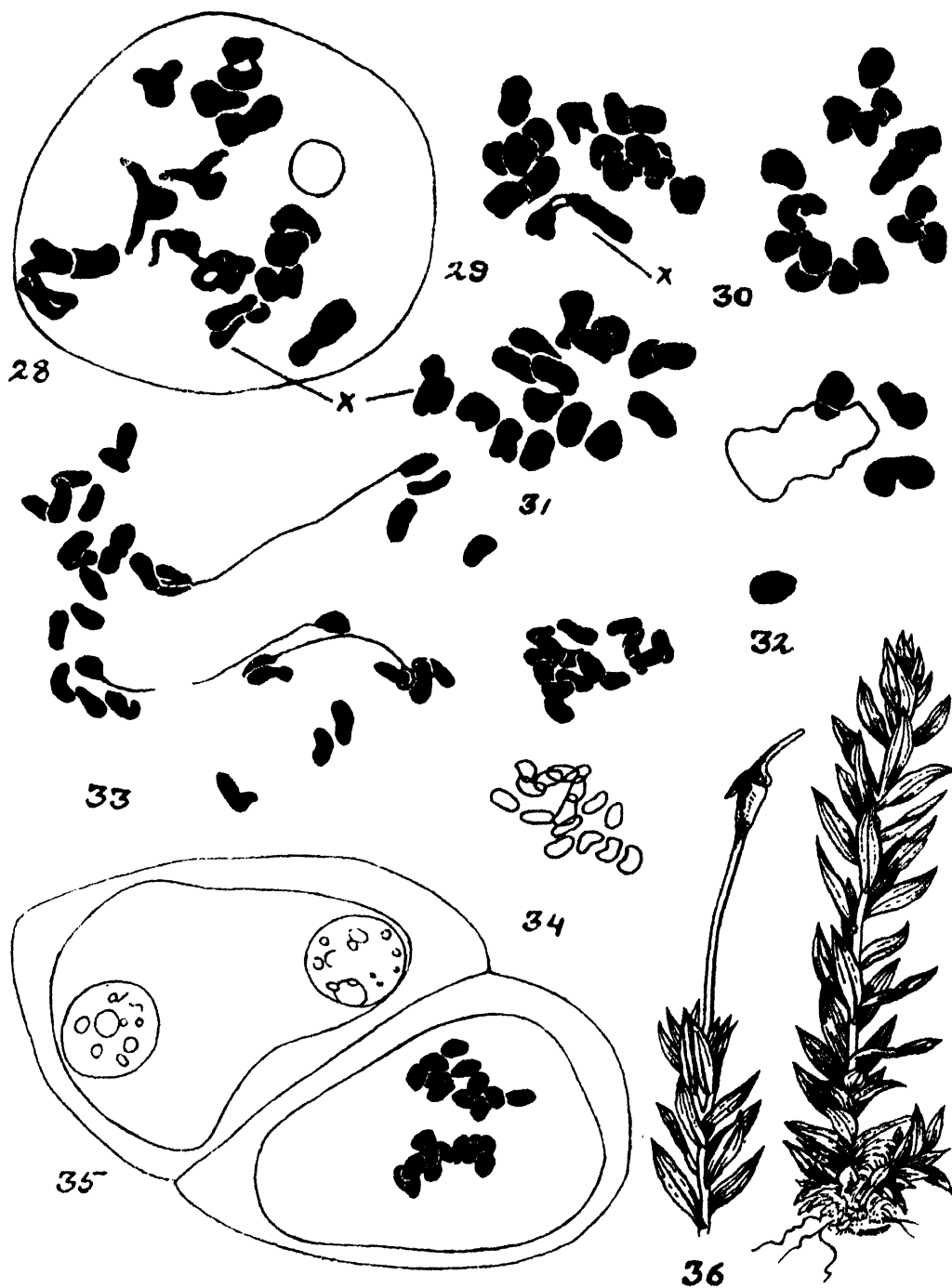
The base of the capsule, contributed by the seta, the apophyses, consists of a mass of polygonal cells full of chloroplasts: a few stomata are found scattered on the surface (Fig. 24). The amphithecial cells also contain chloroplasts when the capsule is young.

(d) *Morphology of the mature capsule*.—The mature capsule is subcylindric, 2 mm. long, brownish red in colour, exerted on a long thin seta, red in colour, 8 to 10 mm. long. Columella attached to the base of the operculum. The operculum carries a part of the columella with it at the time of dehiscence. The operculum is conical with long oblique beak, calyptra hood-shaped, covering almost half of the capsule (Figs. 25, 25a). Peristome teeth are sixteen in number, each of which divides to form thirty-two long, membranous, filiform branches, reddish brown in colour, bearing small rod-like thickenings throughout their surface except at the base (Figs. 25, 27). The peristome branches lie entangled with one another, spirally twisted, almost covering the mouth of the urn when dry. In presence of moisture the peristome teeth uncoil and become straight opening the mouth of the urn.

(e) *Meiosis*.—Study of reduction division of the spore mother cells reveals the presence of sixteen bivalent chromosomes at diakinesis and metaphase I (Figs. 28–31). The bivalents are mostly rod-shaped, excepting two or three, which are subspherical (with two narrow chromatin extensions at either ends), and show size difference during diakinesis. The bivalents undergo further contraction taking up spherical and subspherical forms at metaphase I, and are strongly inclined to clump together. One heteromorphic pair was observed clearly in both diakinesis and metaphase I (Figs. 28, 29, 31). Disjunction of the bivalents are fairly normal during anaphase I (Fig. 34); though irregularities caused by early separation (Fig. 32), late disjunction and non-disjunction of the bivalents resulting into distribution of unequal number of chromosomes at the two poles (e.g. 18 and 14) (Fig. 33) are also not of infrequent occurrence. Second division of meiosis is normal. Diad nuclei with more or less than usual sixteen univalents also undergo normal equational division (Fig. 35) ultimately giving rise to spores with abnormal chromosome complements. This may account for the occurrence of extreme size difference of the spores, ranging from 6.6μ to 18.2μ .

(f) *Spore germination and development of gametophore*.—The spherical brown spores with smooth thin exospore can be divided into three size classes: large, medium and small. Of these, the large sized ones (18.2μ) form 3%, medium sized ones (13.2μ to 16.5μ) 78% and small sized ones (6.5μ to 9.9μ) 19%.

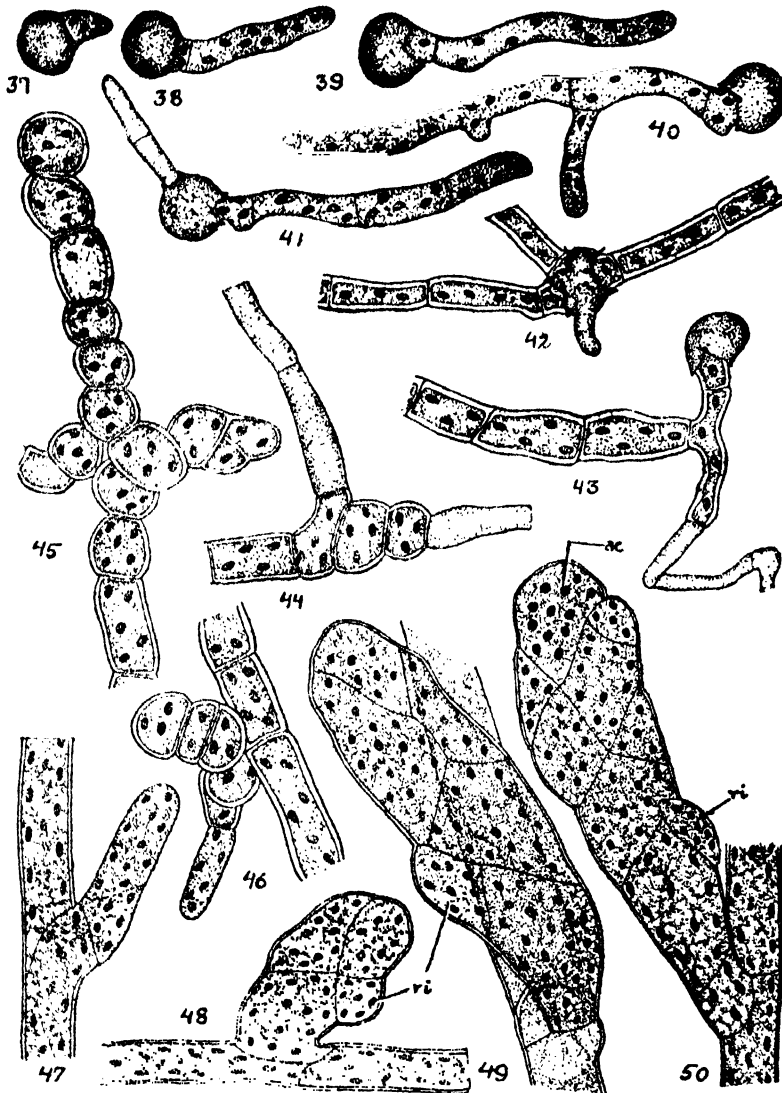
The spores germinate within 3 to 4 days in both solid and liquid nutrient media at room temperature (33°C). A count of the percentage of germination reveals that 50% of the spores from a capsule germinate within the above-mentioned period. Of the remaining 50%, about 20% germinate within a week of inoculation and the



Figs. 28-36. Stages of meiotic divisions in the spore mother cells. Fig. 28. Diakinesis showing 16 bivalents. Figs. 29-31. Metaphase I top view, showing 16 bivalents; heteromorphic pair = X. Fig. 32. Side-view metaphase I, showing early movement of some of the chromosomes. Fig. 33. Late anaphase I showing late disjunction of some of the bivalents and unequal distribution of chromosomes at the two poles (18 and 14). Fig. 34. Metaphase II showing 16 univalents at the two poles respectively. Fig. 35. Anaphase II showing normal splitting of the 11 univalents in one of the diad cells. The other cell is at late telophase II stage. Fig. 36. Mature gametophore with capsule. (Original mags. of Figs. 28-35 = $\times 3,900$; Fig. 36 = $\times 8$.) (Reproduced without reduction.)

rest do not germinate at all in most of the cases. On coming in contact with moisture the spores swell and become turgid and green; marked increase in size by swelling due to absorption of water was not observed (Brown, 1919; Chalaud, 1932; Kachroo, 1954).

On germination the endospore emerges as a papillate protrusion from one side of the spore (Fig. 37) which later develops into a filament (Figs. 38-41). Often germination takes place by development of more than one papillate projections of the endospore simultaneously or successively (Fig. 42). The initial filament (or



FIGS. 37-50. Illustrating germination of spore, development of protonema and gametophore bud. Figs. 37-39. Germination of spore and development of chloronema. Fig. 40. Branching of chloronema. Figs. 41-43. Development of rhizoid from spore and chloronema. Fig. 42. Development of more than one chloronema from a single spore. Figs. 44-46. Formation of terminal and intercalary 'spore-like bodies' or gametes and their germination within 72 hours after supply of moisture. Figs. 47-50. Stages of development of gametophore bud. Fig. 47. Lateral branch with the first oblique wall. Figs. 48-50. Later stages delimiting the three-sided apical cell = *ac* and rhizoidal initial = *ri*. (Original mags. of Figs. 37-46 = $\times 700$; Figs. 47-50 = $\times 1,000$.) (Reduced $\frac{1}{2}$ in reproduction.)

filaments) emerging through a smooth and regular opening of the exospore and containing abundant discoid chloroplasts is soon delimited from the spore by a transverse wall (Fig. 38). With further development of the filament strictly by transverse division of the apical cell, the exospore breaks and rents apart. When the filament is about 3 to 4 cells long, a second filament usually develops directly from the spore, or branches out from the filament (Figs. 41, 42, 43). These are pale green in colour and slender. Under suitable environmental conditions the chloronema develops rapidly and forms a much branched protonema of considerable extent. Branching usually takes place by transverse division of lateral papillate projections originating from an intercalary cell (Fig. 40). The pale green narrow filaments give rise to rhizoidal system by branching; their rate of development is slow in comparison to that of the protonema.

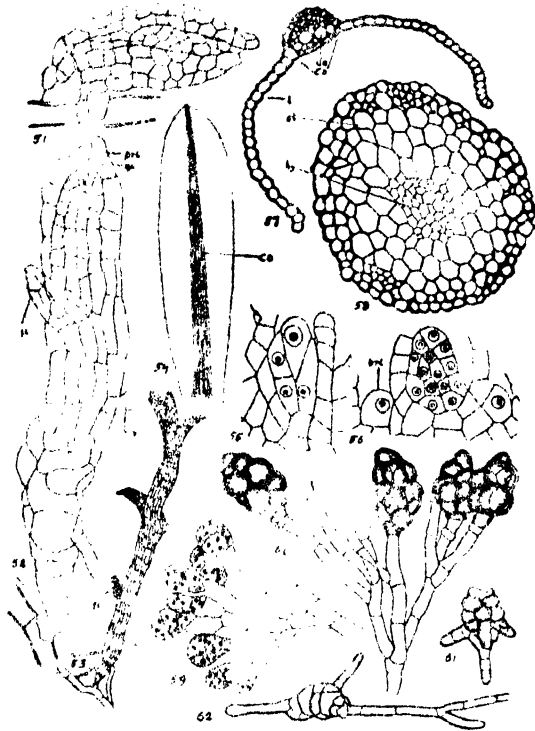
Under suitable environmental conditions the protonema shows definite phototropic response. The chloronema growing horizontally on the surface of the agar medium gives out branches which grow upwards towards the source of light, whereas the pale green, narrow rhizoidal filaments are found to be negatively phototropic growing vertically downwards through the medium. Allsopp and Mitra (1956) recently report the occurrence of similar phototropic response of the protonema in some mosses including the allied genera *Tortula*.

In contrast to the spore germination types falling into different categories in *Funaria hygrometrica* (Schoene, 1906), *Physcomitrium turbinatum* (Case and Mayer, 1950) and *P. pyreforme* (Kachroo, 1954), a uniform type of germination has been observed in the present case, e.g. the chloronema appears first followed by the rhizoid.

In cultures of protonema on Benecke's nutrient agar, Benecke's nutrient solution was added in small amount at intervals of one week to maintain an adequate concentration of the nutrients and water content of the agar (Mayer, 1942). This helped in the development of the gametophore bud. In liquid culture media the development of protonema and appearance of gametophore bud were not observed to take place. Under condition of draught caused by stopping the supply of moisture in cultures of protonema on Benecke's nutrient agar, terminal and intercalary cells of the chloronema undergo rapid transverse divisions giving rise to portions with small rectangular cells. These cells ultimately swell up to almost twice their original breadth and with thickening of their wall give rise to terminal or intercalary chains of spore-like bodies (Figs. 44, 45) which may get detached or remain intact. Restoring them to suitable supply of moisture, these bodies gave out papillate projections (Fig. 46) which develop into normal long celled protonemal branches within 72 hours.

The development of the gametophore bud takes place from the protonema four months after the inoculation of the spores. A two to three celled lateral branch of the protonema increases in breadth and becomes somewhat pear-shaped (Figs. 47, 48). This is followed by formation of strongly oblique division walls of the two distal cells (Fig. 49). The apical cell by further formation of oblique division walls gives rise to the typical inverted pyramidal apical cell of the gametophore having three cutting faces (Figs. 50 *ac*, 52 *ac*, 55). The inner cell is divided unequally by the first oblique division (Fig. 48). The smaller segment functions as the rhizoidal initial. The larger segment together with the three to four segments above (the distal cells not contributed by the three-sided apical cell) undergo no further division throughout the process of gametophore development (Fig. 52). Development of the axis of the gametophore takes place from the apical cell in the manner described above. Generally the leaf initial is differentiated by a periclinal division of the third or fourth segment cut off by the apical cell and gives rise to the lamina of the leaf. The first formed leaf is usually small and imperfect (Figs. 52, 53 *fl*). The young leaves elongate rapidly and become closely imbricated covering the growing tip of the axis (Fig. 51). This is followed by elongation of the axis

starting from the base, thus separating the leaves which show the typical tristichous arrangement (Fig. 53).



Figs. 51-62. Fig. 51. Gametophore bud with closely imbricated young leaves covering the axis. Figs. 52, 53. Gametophore after elongation of the axis; showing the apical cell = *ac*; primordial leaves = *prl*; the first formed leaf = *fl* and rhizoids = *r*. Fig. 54. A leaf; costa = *co*. Figs. 55-56. L.S. of the apex of the axis showing inverted pyramidal apical cell and the branch initials = *bri*. Figs. 57-58. T.S. of mature leaf and axis; lamina = *l*; costa = *co*; outer cells = *de*; hydroid = *hy*; sterids = *st*. Figs. 59-60. Young and mature brute bodies or propagula. Figs. 61-62. Germination of propagula and development of chloronema. (Original magns. of Figs. 51, 52, 55 and 56 = $\times 1,000$; Fig. 53 = $\times 140$; Figs. 54 = $\times 40$; Figs. 57-58 = $\times 550$; Figs. 59-62 = $\times 250$.) (Reduced $\frac{1}{2}$ in reproduction.)

(g) *Regeneration of the gametophore*.—Regeneration of the gametophyte takes place by various means:

1. *Brute bodies* (Van Der Wijk, 1932).—In mature plants one or more filamentous branches arise from a superficial cell (or cells) of the axis at the axil of a leaf. These branches become 8 to 10 cells long and give out branches of the second and third order. Two or three cells including the apical cells of these branches increase in volume, become deep green in colour with dense cytoplasmic contents (Fig. 59). These portions by further irregular transverse and vertical divisions form multicellular spindle-shaped bodies which at maturity become yellowish brown in colour, round in shape and thick-walled (Fig. 60). The mature bodies are usually made up of 12-14 cells, the length and breadth varying from $73-133\mu$ and $34.4-43\mu$ respectively. The cells on coming in contact with moisture give out papillate projections which give rise to much branched protonema and rhizoidal branches as in the case of the spores (Figs. 61, 62). The Brute bodies germinate readily while still lying in penicilloid clusters at the axil of the leaf. In culture media, they germinate within forty-eight hours. Bartram (1949) has reported the presence of Brute bodies (propagula) in *B. cruegeri* and *B. orizabensis*.

2. Regeneration of injured or uninjured leaf and axis of the gametophore by the development of protonema takes place readily in presence of moisture. The pattern of protonema development is, however, different. The rhizoidal filaments develop first from the cells of the mature axis, superficial cells of the costa, cells of the lamina and their margin, and especially from the injured leaves and axis. These pale green narrow rhizoids with oblique transverse walls give out stout chlorophyllose branches forming the protonema.

3. Sometimes the rhizoids of mature plants give out stout, deeply chlorophyllose branches forming the secondary protonema.

4. Usually after a long period of drought followed by a few showers, the apex of the axis of the mature plants elongates and bears partially developed leaves (Fig. 63). These portions usually get detached from the mother plants and give rise to new gametophores.

5. Regeneration of capsule and seta was not observed to take place both in artificial cultures and under natural conditions.

SUMMARY

The life-history of *Barbula indica* Bridel. has been studied in detail. The erect yellowish-brown gametophores are laxly foliose at the base, with definite tristichous arrangement of the leaves near the apex. Leaves are lanceolate with recurved margins and strongly developed costa.

Transverse sections of the axis show a narrow thin-walled central cylinder (hydroid) surrounded by thick-walled cortical cells (steroids). T.S. of leaves show that the costa is composed of four deuter cells surrounded by substereid cells. The lamina is one cell in thickness.

The mature capsule is subcylindric, exerted on a long thin seta; operculum conical, carries a part of the columella at the time of dehiscence. Calyptra hood-shaped. Peristome teeth thirty-two, long membranous, reddish brown, filiform branches are with rod-like thickenings on the surface, spirally coiled covering the mouth of the urn.

Plants are dioecious, development of the sex organs is typical as that of the mosses. The club-shaped antheridium and archegonium with a massive venter and long narrow neck have distinct operculum organized at the tip.

The sporogonium develops from two apical cells with three cutting faces organized by the zygote where the first transverse division is followed by oblique divisions of the two segments. The hypobasal apical cell contributes to the seta and epibasal one to the capsule. Spores show size difference (6.5μ – 18.2μ).

Sixteen bivalent chromosomes were observed to be present in the dividing spore mother cells. Presence of one heteromorphic pair was observed. Disjunction of bivalents was fairly normal though irregularities, e.g. early separation and non-disjunction, were also noted. Second division of meiosis was normal.

Development of gametophore bud from the protonema takes place under suitable environmental conditions within four months of inoculation of the spores on Benecke's agar medium. On germination the chloronema develops first followed by the rhizoids.

Various modes of regenerations of the gametophore have been described.

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ON THE BIOLOGY OF *AURICULARIA AURICULA-JUDAE* (LINN.)
SCHROET. CAUSING ROT IN ELDER (*SAMBUCUS NIGRA* L.)

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(Communicated by G. P. Majumdar, F.N.I.)

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INTRODUCTION

Auricularia auricula-Judae (Linn.) Schroet., the common Jew's ear fungus, is fairly distributed throughout the world and is found in both temperate and tropical regions. In Great Britain it is known to occur commonly on elders but more rarely on beech, elm, oak, walnut, willow, holly and *Berberis arcuata* (Rea, 1922). Besides *Sambucus nigra*, Poetsch (1877) lists a number of other plants, viz. *Ailanthus glandulosa*, *Elaeagnus angustifolia*, *Morus nigra* and *Robinia pseudacacia*, on which it occurs. In India, it occurs commonly on logs of *Shorea robusta* and dead stumps and branches of *Psidium guyava* and *Ficus religiosa* (Banerjee, 1947). Secretan (1833) divided the fungus into three varieties of which var. *B. caraganae* (Pers. Syn., p. 625) was found growing on a large dead branch of *Lycium barbarum* while the other two varieties were always found on *Sambucus nigra*. In Great Britain its fructifications are abundantly formed during the cold, wet months of November to February. It is often found in great quantity growing entirely saprophytically on trunks and branches without attacking the living trees but it is sometimes supposed to grow parasitically on elders (Ainsworth and Bisby, 1945). Thumen (1876) found it in autumn on two new substrata, viz. *Acer negundo* and *Hibiscus syriacus*, and mentioned that in both cases the fungus grew luxuriantly on the healthy host plant. Tunstall (1923, 1925, 1940) in Cachar, Assam (India), described it as the commonest wound parasite killing the branches of the tea plants and in time extending even to the roots, but he did not give any experimental evidence in support of his statement. Geneause and Kuenzel (1939) isolated it from the rotted heartwood of three living black walnut (*Juglans nigra*) trees but its etiological complications had not been established. Though a great deal of research work has been carried out on this fungus, its activities as a parasite on elder (*S. nigra*) and the amount of damage it causes in the wood are not yet completely understood. With this end in view, an investigation into the nature of the disease, the amount of damage in the wood caused by it and the life-history of the fungus in culture have been undertaken. The data so far obtained are given in the following pages based largely from observations in the laboratory as well as in the field.

THE ELDER: ITS ECONOMIC IMPORTANCE

The common elder tree (*Sambucus nigra* L.), the natural host of the Jew's ear fungus, is widely distributed in Great Britain, central and southern Europe, thriving mostly in wasteland and in shady places in woods on many kinds of soil. Until recently, foresters and farmers regarded it as a plant of little value and as such it was usually heavily cut down and destroyed by burning by the landowners. Although the flowers, fruits and bark of elder have been long recognized in medicine for curing various troubles, the plant has now practically lost its reputation as having considerable therapeutic value. In the country districts of Great Britain,

the berries are commonly used to manufacture elderberry wine. Recently, Metcalfe (1948) has pointed out the economic importance of elder and showed that its wood is particularly suitable for the preparation of watchmakers' pegwood and high grades of charcoal. The sticks of elder pith are usually used in botanical laboratories for holding small specimens. Watch and other instrument makers use it for manipulating small objects. It was only during the last World War that the preparation and marketing of elder pith were undertaken in Great Britain, as it was thought to be unwise to ignore the potential uses for any raw material available in this country. Metcalfe further advocates the cultivation of elder for manufacture of pith, high grades of charcoal and timber suitable for making delicate objects and this might be undertaken profitably as a small-scale industry.

HISTORY AND SYNONYMY

Auricularia auricula-Judae, the Jew's ear or Judas' ear, was well known and described at least as far back as the end of the sixteenth century. Gerarde (1597, 1633) mentioned and figured this fungus as '*Fungus Sambicinus*, sine *Auricula Judae*, Iewes eares'. Later, Micheli (1728) described and figured the fungus as *Agaricum Auriculae forma*. It was mentioned by Linnaeus (1753) as *Tremella Auricularia*. Persoon (1801) described the fungus under the name *Tremella Auricula-Judae* but Fries (1822-23) transferred it to the genus *Exidia* under the tribe *Auricularae* and described it as *Exidia Auricula-Judae*. Berkeley (1860) re-described the fungus under the generic name *Hirneola* by which it was known for a long time. Schroeter (1889) transferred it to *Auricularia* and this appellation had come into common use (Rea, 1922; Ainsworth and Bisby, 1945). Because of its widespread distribution, its ability to grow on many kinds of decayed wood and its great variety of shapes, sizes and colours depending on its age or the dryness or moistness of the surrounding atmosphere, it has been described from time to time as new species under various names as the following list of synonyms indicates.

Barrett (1910) records the following: *Tremella Auricularia* L. Sp. Pl. 1157, 1753. *Peziza Auricula* L., Syst. Nat. ed. 12, 2: 725, 1767. *Merulius auricula* Roth., Germ. 1: 535, 1788. *Peziza Auricula-Judae* Bull., Champ. 1: 241, 1791. *Tremella Auricula-Judae* Pers., Obs. Myc. 2: 93, 1799. *Auricula sambucina* Mart., Fl. Crypt. Erl. 459, 1817. *Exidia Auricula-Judae* Fr., Syst. 2: 221, 1822. *Auricula ampla* Pers., in Freyc., Voy. 177, 1826. *Exidia auricula* Wallr., Fl. Crypt. 2: 559, 1833. *Exidia ampla* Lév., Ann. Sc. Nat. Bot. III, 5: 159, 1846. *Hirneola auricula-Judae* Berk., Outl. 289, 1860. *Hirneola ampla* Sacc., Syll. 6: 765, 1888. *Auricularia Auricula-Judae* Schröt., Krypt. Fl.: Schles. 3: 386, 1889. *Auricula ampla* Kuntze., Rev. Gen. 2: 844, 1891. *Auricula Auricula* (L.) Underwood., Mem. Torrey Club. 12: 15, 1902.

Barrett also considers that in all probability *Auricularia auriformis* (Schw.) Earle is the same fungus and, if it proves to be the same, several more synonyms should be added to the list.

According to Moller (1895) both *Laschia delicata* Fr. and *L. tremellosa* Fr. are synonyms of *A. auricula-Judae*. He found that it is common in Brazil.

THE SPOROPHORE

(Plate XXVIII, figs. 1 and 2)

Description

Fructifications.—Sessile and attached by a point or sub-stipitate; erumpent; single or in groups; often imbricate; at first cup-shaped then becoming irregularly lobed or folded, sometimes auriform or conchiform; soft, gelatinous or cartilaginous, semi-transparent, trembling when moist; dimension variable, about 1.5-9 .1

-5 cm. when fresh; margin at first entire but somewhat lobed in older specimens, curling over the hymenium on losing moisture.

Upper surface.—When young smooth, becoming tomentose with fine, short, sub-bulbous hairs with age; colour varies from Hay's russet to Liver brown with greyish hairs which change to Olive brown, Benzo brown, Cinnamon drab or Fawn colour on drying, finally becoming Fuscous; irregularly veined and becoming wrinkled on losing moisture.

Context.—Gelatinous or cartilaginous, becoming hard and horny on drying; at first whitish; about 1-2.5 mm. thick.

Hymenial surface.—At first smooth, then venoso-plicate with age; colour varied, Hay's russet or Greyish, Fawn colour to Army brown, often with a lilac tinge, becoming Fuscous or Fuscous black on drying, shining or dull with a whitish bloom.

Basidia.—Elongated, cylindrical to somewhat fusiform, often slightly flexuous, transversely septate, forming a compact hymenium; sterigmata four of unequal lengths, each developing from a cell of the basidium; dimension about 55-85 (100) \times 5-7-10 μ .

Spores.—Hyaline; thick-walled; smooth; oblong, cylindrical or curved; dimension about 17-22 \times 7-10 μ .

Tissue differentiation.—In a cross-section of a mature fructification, the following regions can be differentiated: (1) a compact hymenial layer, about 80-100 μ thick, consisting of elongated basidia; (2) an intermediate hyaline zone making up the major portion of the context; about 1,000-2,000 (2,500) μ thick when moist; consisting mainly of hyaline, much branched, stainable hyphae embedded in a matrix formed of hyaline, non-stainable, gelatinized hyphae, mostly 1-1.5 μ , sometimes up to 2.5 μ across, with numerous simple clamp-connections, running more or less parallel but becoming closely interwoven below the hymenium forming a brownish zone, about 100 μ wide; (3) an outer dark brownish compact zone of densely interwoven hyphae from which the hairs arise; variable in thickness; numerous dark brown, thick-walled, flexuous or contorted, isolated upright hyphae, about 7.5-10 (12) μ wide present; hairs thick-walled, septate, sub-bulbous, hyaline to pale yellow, pointed, rounded or truncated at the tip, about 5-8 μ wide.

The colours in all cases are according to Ridgway (1912). The fruiting body in itself is very characteristic and readily recognized in the field. The imbricate habit of growth, soft, gelatinous or cartilaginous, semi-transparent, irregularly lobed, sometimes conchiform or auriform and sessile fruiting bodies are distinctive features. When young and in a moist condition, it is frequently turbine-shaped but with age in a rainy weather, it is greatly extended in size, becomes lobed and somewhat undulated at the margin and the lobes lying over one another.

DECAY OF ELDER

A. *Material*

The materials were collected in February, 1950, in the form of freshly cut infected trees of elder (*S. nigra*) with fructifications of *Auricularia auricula-Judae* from elder bushes in Hopetoun Estate, near Edinburgh, Scotland. The situation was somewhat shady, cool and damp and as such the fructifications of the fungus were found growing luxuriantly on decaying trunks and older branches of living trees bearing numerous young upright shoots. The trees were carefully selected, cut down and macroscopically examined in the field to note as far as possible the progress of decay in the wood. For further study, small sections from different regions of the diseased trunk and branches were selected, brought into the laboratory, examined and studied while in a fresh condition. Microscopic examinations of the rotted areas showed abundant mycelium within the tissues of the host.

B. *Macroscopic characters of the rot*

The symptoms that are externally visible are the presence of numerous fructifications (Pl. XXVIII, fig. 1) of the fungus bursting open through the decayed or cracked bark of the trunk and rotting of the peripheral portions of the wood where the decay has advanced to a considerable extent. The bark of the decaying wood separates easily and often patches of whitish mycelium are present in between the bark and the decayed wood. With the exception of the green shoots, the fructifications are found all over the trunk. Cross-sections of the infected wood show that the early stages of attack are marked by the formation of small, incipient rot pockets at the periphery of the wood being bounded internally by a narrow, water-soaked, light yellowish or pale brownish invasion zone (Pl. XXVIII, fig. 3). These gradually become broader and lighter in colour as the attack proceeds and may finally coalesce so that in an advanced stage of decay both sapwood and heartwood become light, soft, spongy and easily break up into sections. The bark comes away easily. In an advanced stage of decay, a cross-section of the diseased trunk shows a hollow central region (Pl. XXVIII, figs. 4 and 5) surrounded by the partially decayed wood which is somewhat whitish and crumbling. The rots showing various stages of decay appear as brownish or pale coloured irregular areas (Pl. XXVIII, fig. 4), with small pockets which may or may not contain white material and in others these are reduced to a mess of whitish or pale coloured fibres. Finally, the wood crumbles easily and thin sheets of conspicuous whitish mycelium are found in the shrinkage cracks formed along the grains of the wood (Pl. XXVIII, fig. 6). Eventually, the rotten wood falls into small flakes. The rot is confined to the trunk and older branches and does not extend into the young green shoots.

C. *Microscopic details of the rot*

In order to study the character and distribution of the mycelium in the infected wood, small pieces of sound and diseased wood were sectioned both freehand and with the microtome. The materials were first softened with equal parts of alcohol, glycerine and water and then transverse, radial and tangential longitudinal sections, 15–18 μ thick, were cut. Freehand-sections were, in general, found to be sufficiently thin to show the presence of hyphae in the tissue-elements but for detailed examination and successful photomicrography under high magnifications sections prepared with the microtome were particularly suitable to answer the requirements.

Differential staining was made of the sections by the methods described by Hubert (1922) and Cartwright (1929). Of these, Cartwright's safranin and picro-aniline blue were found to be most positive and satisfactory in rendering the hyphae visible and easy to manipulate. Since this technique has got certain objectionable features, such as the hyphae often become contracted and distorted and the precipitation of the granular masses of aniline blue on the hyphae due to over-heating, the modified method of Proctor (1941), by using dilute concentration and reduced amount of heat, was found to be very effective. The sections were ultimately dehydrated and mounted in balsam.

The distribution of the hyphae in the decayed wood is fairly uniform (Pl. XXXI, figs. 21–25). At first the hyphae develop mainly in the medullary rays and to a certain extent in the vessels; in the wood fibres they are comparatively less numerous. The hyphae, which are all hyaline, vary greatly in thickness, some are wide with granular contents (2.5–3.5 μ across) and others quite narrow (1.2 μ wide). The vessels are often filled up at places (Pl. XXXI, figs. 21, 23, 25) with a web of wider hyphae which are sparingly branched and with frequent simple clamp-connections (Pl. XXXI, fig. 26). In the narrower hyphae the clamp-connections are less numerous. The hyphae at first pass from cell to cell mainly through simple

pits of the thick-walled ray cells (Pl. XXXI, fig. 28) and simple or inconspicuously boarded pits of the elements of the vessels (Pl. XXXI, figs. 24, 26, 27). Later they directly penetrate the cell-walls by fine thread-like pegs or outgrowths, at length enlarging the bore-holes (Pl. XXXI, figs. 29-31). In an advanced stage of decay the hyphae become numerous and tend to become more abundant in the medullary rays and in the wider lumina of the irregularly arranged moderately thick-walled wood fibres with rather infrequent, slit-shaped pits which are apparently simple. Much branched hyphae often completely fill up the lumina of the fibres (Pl. XXXI, fig. 22). The penetration of the walls of the wood cells by the hyphae either shows no diminution in hyphal diameter (Pl. XXXI, figs. 29, 31) or in case of wider hyphae their apices become attenuated into fine points (Pl. XXXI, fig. 30). During penetration the narrow hyphae are often clearly visible within the wider bore-holes (Pl. XXXI, fig. 30) through which they pass. After penetration the hyphae thicken till they reach their mature width while the portion within the bore-holes remains exceedingly fine. The bore-holes, round or oval, become very numerous and are about 1-2 μ across (Pl. XXXI, figs. 29-31). Sometimes they coalesce to form an irregularly shaped hole. The bore-holes have smooth, moulded contours and are not irregularly ruptured or splintered. This indicates clearly that the dissolution has been caused by a solvent originating from a central point. This unmistakable stamp of chemical dissolution of the cell-wall by enzymic activity of the fungus supports Proctor's (1941) theory of cell-wall penetration. At places, the bordered pits are eroded and become somewhat indistinct and transparent. The fibres become more conspicuous with spiral shrinkage cracks which radiate from the bore-holes and pits (Pl. XXXI, figs. 22, 23). Similar spirally orientated cracks are sometimes found on the disintegrating walls of the vessels. Yellowish-brown staining of the cell-walls may develop in the vessels and medullary rays in an advanced stage of decay. A yellowish gum-like material is also present in the decayed wood.

D. *Micro-chemical studies*

Results of micro-chemical staining for lignin and cellulose in the sound and partly decayed wood are recorded here. This has been done by staining the sections of wood with phloroglucin-HCl for lignin and chlor-zinc-iodine for cellulose. The combination stain, Bismarck brown and Gentian violet, which differentiates un-lignified (brown staining) and lignified (violet staining) structures, has also been tried. Various other micro-chemical stains have also been employed, most of which agree closely with the changes indicated by the use of the above-mentioned stains. Although these micro-chemical stains are not infallible indicators of the chemical changes that take place in the wood, yet these are commonly used in such work to indicate the presence of lignin and cellulose and the results are presented in Tables 1 and 2.

From Tables 1 and 2 it will be evident that phloroglucin-HCl and Gentian violet indicate the presence of lignin in the primary and secondary walls of all the elements in the normal wood. Chlor-zinc-iodine and Bismarck brown stain the secondary walls blue and yellowish brown respectively indicating the presence of cellulosic materials in the wood elements. In the partially decayed wood the chemical changes indicated by the stain-reactions clearly show that primarily there is gradual delignification of the highly lignified parts of the secondary walls and as such the residual cellulosic materials show more intense staining reaction. The reactions do not indicate clearly the removal of cellulosic materials in the early stages of decay in the wood. It can, however, be said that the fungus attacks lignin more intensely than cellulose.

Sections of normal and decayed wood corresponding to those used for micro-chemical stains have been placed on slides and treated with 72% sulphuric acid as suggested by Ritter (1925). Immediately in the sections of both normal and

TABLE 1

Results of staining for cellulose and lignin in normal and partially decayed wood of elder with Bismarck brown and Gentian violet respectively

Elements	Normal wood	Decayed wood
Vessels ..	Primary walls deep violet; secondary walls partly light violet but mostly yellowish brown around the lumen.	Primary walls violet; secondary walls mostly light yellowish brown.
Fibres ..	Primary walls deep violet; secondary walls mostly light violet, deeper near the middle lamella, mostly yellowish brown around the lumen.	Primary walls violet; secondary walls mostly pale violet but partly brownish, often deep brown at places.
Ray cells ..	Primary walls violet; secondary walls mostly light violet but partly yellowish.	Primary walls mostly light violet but light brown at places; secondary walls mostly pale brown or brown but occasionally pale violet.
Wood parenchyma ..	Primary walls deep violet; secondary walls light violet but partly yellowish.	Primary walls light violet; secondary walls partly light brown at places.

TABLE 2

Normal and partially decayed wood of elder treated with chlor-zinc-iodine and phloroglucin-HCl for staining cellulose and lignin respectively

Elements	Normal wood	Decayed wood
<i>Cellulose (chlor-zinc-iodine)</i>		
Vessels ..	Secondary walls often faint blue around the lumen.	Secondary walls faint blue.
Fibres ..	Secondary walls faint blue particularly around the lumen, often partly so.	Secondary walls partly or mostly light blue, occasionally in groups partly or entirely blue.
Ray cells ..	Secondary walls partly faint blue.	Secondary walls mostly light blue or blue, primary walls light blue at places.
Wood parenchyma ..	Secondary walls partly faint blue.	Secondary walls pale blue at places.
<i>Lignin (phloroglucin-HCl)</i>		
Vessels ..	Primary walls red; secondary walls light red or pale pink to almost colourless.	Primary walls red to pink; secondary walls pink to colourless when broken down.
Fibres ..	Primary walls deep red; secondary walls light red, often pale pink near the lumen.	Primary walls red; secondary walls light red but somewhat colourless near the lumen.
Ray cells ..	Primary walls deep red; secondary walls red to light red.	Primary walls red; secondary walls light red to pink.
Wood parenchyma ..	Primary walls deep red; secondary walls red.	Primary walls red; secondary walls light red.

decayed wood, the cells swell considerably and there is much distortion throughout the tissue-elements. The middle-lamellae are torn apart, the elements are isolated in groups and the medullary rays become much convoluted in appearance. This violent action during the process indicates the presence of considerable amount of cellulose in the cell-walls of both normal and decayed wood. The swelling of this cellulose preceding dissolution forces all the elements apart in the wood. Though not conclusive in itself, this test at least indicates the presence of much cellulose in the wood partially decayed by the fungus.

CULTURE STUDIES

(a) *Spore and tissue cultures*

The initial cultures were made by the use of spores and from portions of the infected wood. Copious spore-deposits were obtained within a few hours from a fresh sporophore on 2.5% malt agar contained in the lower lid of a Petri dish, while a trimmed rectangular piece of the sporophore was fixed excentrically with the hymenial surface downwards to the inner side of its upper lid, and placed inside a moist chamber. The spores, thus deposited, were transferred aseptically to culture tubes containing malt agar. The cultures were also made from partially decayed wood. Small bits of infected wood were washed several times with sterile distilled water and flamed lightly in order to kill as many superficial spores as possible from the surface. A sterile scalpel was used to cut away the exterior portions and bits of wood pieces thus exposed were carefully inserted in malt agar tubes. These were then kept in an incubator at 23°C. in darkness. In the course of a week or so mycelia grew out and these served as stock materials for sub-cultures.

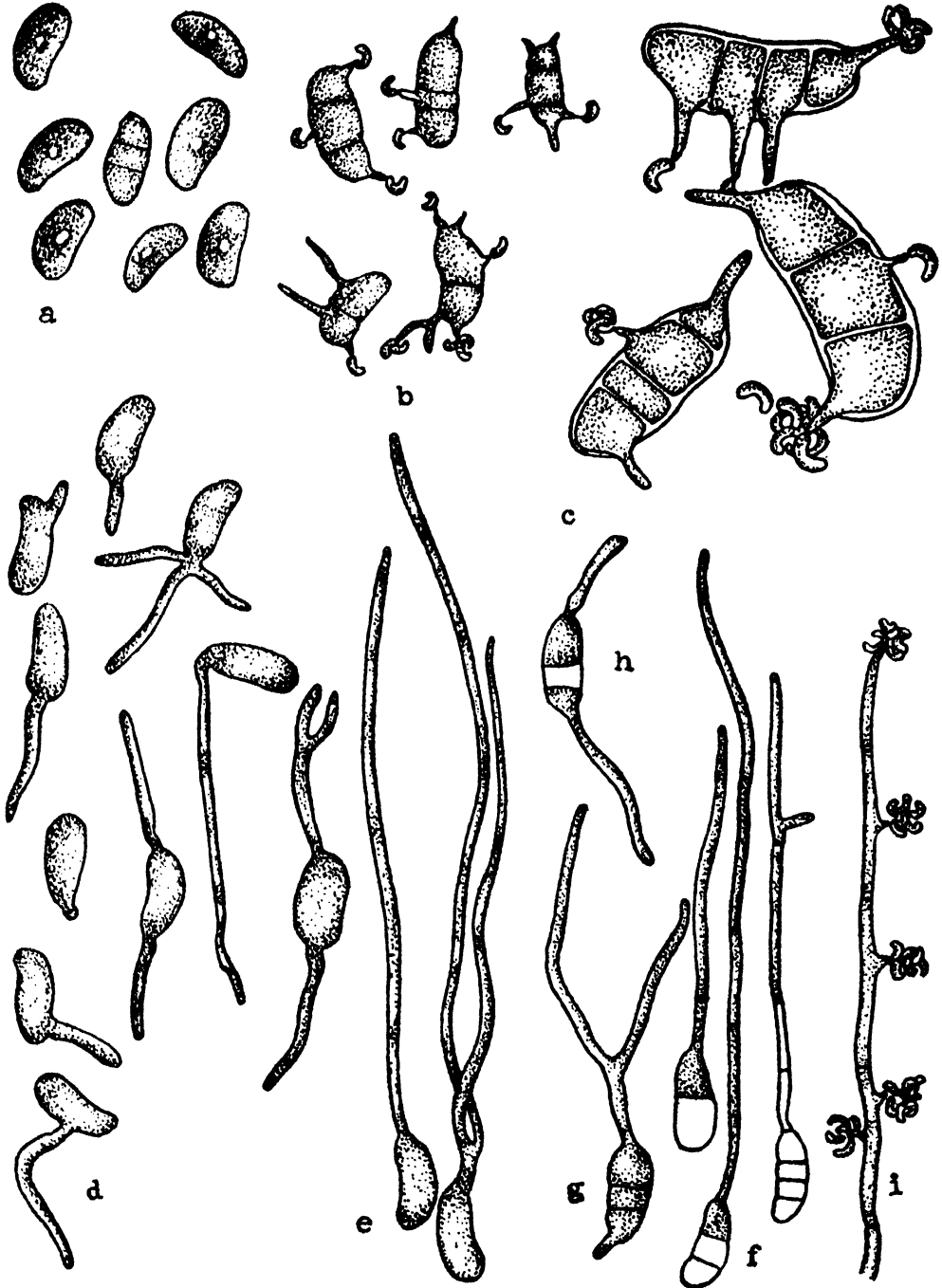
(b) *Spore-germination*

(Plate XXX, figs. 15-17)

The basidiospores were obtained from fresh sporophores found on elder. The fruiting bodies continued to cast their spores for 48-72 hours and spore-deposits were obtained on sterile slides contained in a Petri dish. Germination tests were made with these spores in hanging drops of water in the usual way and also upon 2.5% of malt agar and potato-dextrose agar in Petri dishes. Different samples of distilled water and tap water were tried. These were kept in darkness at constant temperatures of 18°C. and 23°C. in incubators and in diffused light and room-temperature (23°C.-25°C.).

On all agar media germination occurs within 12-18 hours under all the different conditions of light and temperature. The room-temperature and diffused light have been found to be more suitable for direct and rapid germination. The one-celled spores (Text-fig. 1, *a*) germinate by producing long, thick and stout germ-tubes only either at the opposite ends or from the sides (Text-fig. 1, *d*, *e*). When one germ-tube is produced, usually its protoplasm migrates into the tube and with its gradual advance 1, 2 or 3 septa are successively laid down within the empty spore-case (Text-fig. 1, *f*). This process may be continued even in the newly formed hypha where a few empty hyphal cells can be differentiated at the base from the terminal portion which is filled up with densely granular protoplasm. A spore may form two germ-tubes (Text-fig. 1, *d*) simultaneously at opposite ends, of which one soon takes the lead and the whole protoplasm of the undivided spore retracts from the opposite side and migrates into the more actively growing germ-tube followed by successive formation of septa towards the opposite end within the original spore-wall. Sometimes the spore is at first divided by a wall into two cells, each producing a separate germ-tube. Occasionally, when two germ-tubes are produced the protoplasm splits up in the middle within the spore-case and as it

moves into the germ-tubes two walls are laid down within the spore forming an empty cell at the centre (Text-fig. 1, *h*). Subsequent growth gives rise to luxuriant mycelium in all cases.



TEXT-FIG. 1. Spores and spore-germination in *Auricularia auricula-Judae*.

In water two kinds of germination have been observed. At 18°C. the spores usually become divided into 2-4 cells from each of which one or more short or slender outgrowths are produced. These give rise to single or clusters of sickle-shaped or hoof-like conidia at the apices (Text-fig. 1, *b*, *c*). At 23°C. the spores not only bear conidia from their sides but some of them germinate by producing much branched germ-tubes bearing clusters of conidia at the apices of short lateral branches (Text-fig. 1, *i*). Occasionally, however, one or two cells of the spores germinate by germ-tubes while others produce only conidia. Brefeld (1888) was the first to study the germination of the spores in *Auricularia auricula-Judae* in water and culture solution but he did not mention about the exact conditions under which the spores germinated. He stated that by using a richer and more concentrated culture solution, the spores divided rarely and produced luxuriant mycelia. In the present investigation similar results were obtained on agar media under different conditions of light and temperature and the spores also remained undivided but most often each was divided into 2 to 4 cells. In water, on the other hand, he obtained germination by the production of conidia on short side branches from each cell of the divided spore but in the present study long, much branched germ-tubes bearing clusters of conidia at the tips of short lateral branches could be obtained as a result of germination in water at 23°C. It can, however, be said that low temperature and water favour germination by the production of conidia on short outgrowths from the side of the spores while in dilute culture solution and in water at a comparatively higher temperature long germ-tubes bearing conidia can be obtained. Solid nutrient media, on the other hand, inhibit the production of conidia and favour direct germination to form luxuriant mycelium under all conditions.

(c) *Mycelium in culture*

The mycelium of *Auricularia auricula-Judae* isolated from the decayed wood as well as the polysporous mycelium were grown in culture and their characteristics studied on the different media, viz. potato-dextrose agar and 2.5% malt agar. Culture tubes 6" x $\frac{3}{4}$ " in size were used for the study but for determining the rate of growth, they were grown in Petri dishes. All cultures were kept in darkness in different incubators at 18°C. and 23°C. respectively. In order to maintain the experimental conditions as constant as possible small pieces of inocula (about 3-4 mm. in diameter) were taken from young cultures and placed with upside downwards upon the medium in culture tubes and Petri dishes, each containing approximately 20 c.c. and 30 c.c. of the medium respectively. The pH values of the media were so adjusted that after sterilization each had pH 5.2. The colour exhibited by the mycelium has been expressed according to Ridgway (1912) and terms used to describe the texture of the mat are those of Long and Harsch (1918). After a comparative study it was found that the isolates and the polysporous mycelia are identical in cultural and microscopic details.

(1) *Oxidase tests*

The oxidase test as described by Bavendamm (1928) was made by growing the fungus in Petri dishes on 2.5% malt agar containing 0.5% gallic or tannic acid and kept at a constant temperature of 23°C. in darkness. Being a white rot fungus dark brown rings due to the presence of oxidase appeared within 24 hours. The intensity of reaction was, however, mild on gallic acid medium and the diameter of the ring was only 20 mm. even after 168 hours of inoculation. Another method to distinguish white rot fungi from brown rot ones (Preston and McLennan, 1948) was tried by growing the fungus on 2.5% malt agar containing 0.007 per cent Gentian violet. The fungus gave positive reaction and partially bleached the violet colour of the medium below the mat but about 6 weeks' incubation was necessary before definite results could be obtained.

(2) *Cultural characteristics**Auricularia auricula-Judae* (Linn.) Schroet.

(i) *Habit of growth* (Pl. XXX, figs. 13, 14).—On *potato-dextrose agar* the growth was very slow at the beginning and at the end of a week after inoculation thin cottony mycelium appeared over the inoculum with a narrow (1-2 mm. wide) appressed growth over the surface of the slant. In about 10-12 days the inoculum turned sub-felty to felty but the mat proper became differentiated into a central sub-felty zone and an appressed, colourless and fimbriated zone of advance. As the growth advanced the central zone remained sub-felty as before but gradually thinned out towards the upper end of the slant and became raised and cottony to silky towards the lower end. After three weeks the mycelium covered the whole surface of the slant and the mat became felty and homogeneous but with a few raised lumps of mycelium around the inoculum. The surface of the mat gradually turned tough, rough and nodulose at places. A thick skin was ultimately formed and its surface was mostly rugulose but pulverulent at places. At 23°C. the growth was somewhat similar but more rapid and condensed with wider fimbriated advancing zone. The texture of the mat early became cottony-woolly which later turned felty and the silky nature of the mycelium towards the lower end of the tube was more pronounced. The upper end of the mat, instead of becoming thin, was raised and cottony. Finally, tufted and felty patches of mycelium were distributed over the rugulose surface of a thick skin. On *malt agar* at both temperatures this habit of growth was more or less similar to that on *potato-dextrose agar* but in the former it was more vigorous, raised, loose and woolly and the advancing zone was less distinct. The mat proper instead of being rugulose turned pulverulent with age. In all cases colour of the medium turned yellowish in about 10-15 days after inoculation.

(ii) *Colour*.—On *potato-dextrose agar* the mycelium remained white throughout but at 23°C. pale Salmon colour developed towards the lower end of the slant in about a month after inoculation. On *malt agar* pigmentation of the mat became evident in about a month and the shades included Pale Pinkish cinnamon and Light Pinkish cinnamon. At 18°C. colouration was more pronounced and was restricted to the central part of the mat.

(iii) *Rate of growth*.—The rate of growth, both on *potato-dextrose agar* and *malt agar* and kept under identical conditions as stated before, was rather very slow and the daily increment in diameter of Petri dish cultures was determined. The radial growth in all cases was unequal and as such average measurements were taken. On *malt agar* at both temperatures the daily increment of growth varied between 2.5 and 3 mm. while on *potato-dextrose agar* it was only 2.5 mm.

(iv) *Hyphal characters*:

Aerial mycelium.—(a) Wider hyphae, about $2-3\mu$ wide, more or less straight or slightly flexuous, septate, distantly branched, with frequent clamp-connections, clamp-connections often developing into new hyphae, with yellowish brown granular contents; (b) profusely branched, hyaline to yellowish brown, narrower hyphae, about $1-2\mu$ across, usually highly flexuous, sometimes slightly so or more or less straight and with numerous clamp-connections.

Submerged mycelium.—(a) Wider hyphae, about $2.5-5\mu$ across, sometimes up to 7.5μ wide due to swelling, septate, flexuous, profusely branched, irregularly swollen presenting a somewhat gnarled appearance, often constricted, with yellowish or brownish granular contents, branches often clustered, clamp-connections present but infrequent; (b) hyaline, thin-walled narrower hyphae, about $1-1.5 (2.5)\mu$ across, usually slightly flexuous, sometimes straight and unbranched for a considerable distance, usually sparingly branched, with clamp-connections almost at every septum and with highly granular contents.

(d) Production of fruit-bodies in culture

(Plate XXIX, figs. 7-10)

Several unsuccessful attempts were made to obtain fruit-bodies of *Auricularia auricula-Judae* on various agar media kept under different conditions of light, temperature and moisture. Barnett (1937) obtained fertile but atypical fruit-bodies on agar media but he did not mention anything about the exact conditions under which they were formed. Eventually, the medium for developing sporophores recommended by Badcock (1941) was tried with considerable success. Moist sawdusts of beech or spruce with 5% Badcock's accelerator was used in Petri dishes, test-tubes (20×4 cm. and 15×2 cm.) and Badcock's apparatus and the various devices recommended by him (1943) were tried. After sterilization the media were inoculated by the isolates of the fungus and the cultures were kept in darkness at room-temperature (18°C.-21°C.) inside a closed chamber in which a moderately high humidity was maintained. Subsequently, when mycelia made a vigorous growth and covered the surface of the medium or reached the ends of the tubes, all the cultures were removed and placed in strong diffused light on a table about 8 feet from a large window of the laboratory.

Although a dense, white mycelial growth covered the entire surface of the sawdust medium in Petri dishes, fructifications did not appear in any one of them even in cultures 3-4 months old. At this stage the lids of the Petri dishes were removed and the cultures were kept covered under a moist bell-jar near a window at room-temperature. Within 24 hours small fructifications began to appear rapidly at the rim of the Petri dish and they were about 8 mm. in diameter within 48 hours (Pl. XXIX, fig. 8). The fructifications gradually enlarged, at first resupinate, but later became typically reflexed (Pl. XXIX, fig. 7). Sections of such a well-developed fructification revealed a well-developed hymenium with normal basidia and viable basidiospores. Similar fructifications were also obtained in tubes after a period of 2-3 months. When the mycelium completely filled the tubes and became compact, the plugs were removed and each was supported in an inclined position in between two lids of the Petri dishes, the lower being filled with water. A thick pad of absorbent cotton-wool was placed within the mouth of the tube and was never allowed to dry out. Within a few days when vigorous surface growth developed, the pad was gently pushed down so as to touch the medium. Irregular fructifications began to develop within the tube and this irregularity was probably due to limited space within the mouth of the tube for their development. In Badcock's apparatus, fructifications also began to appear within the flask at the rim of the tube (Pl. XXIX, fig. 10) after 3-4 months but their growth was very slow and somewhat irregular. This was in all probability due to limited space within the flask and possibly owing to absence of ultra-violet light or too high a humidity as pointed out by Badcock (1943).

DECAY RESISTANCE TESTS IN THE LABORATORY

The natural resistance to decay of wood block samples of elder was tested in the laboratory. The process in general involved in exposing small sterilized samples of elder to the attack of *Auricularia auricula-Judae* growing in pure culture under controlled conditions and the amount of decay due to fungal attack was estimated by measuring the loss in dry weight. Various methods were suggested and durability tests on different timber were made by previous workers but the method used by the writer was as follows. Small test-blocks were cut and planed to $\frac{1}{2}$ " × $\frac{1}{2}$ " square and 2" long with the long axis parallel to the grain of the wood. In all cases clear material, free from defects were taken. To find out the actual loss from decay, the test-blocks were serially numbered on all faces and were dried in an oven at 60°C. to a constant weight. The blocks were then thoroughly soaked in

distilled water, put in Roux tubes with water at the bottom, plugged and sterilized at 15 lb. pressure for 10 minutes. The object of this was to sterilize the wood blocks in a saturated atmosphere so that little loss of water could take place from the surface of the wood blocks and consequently lesser time was needed for sterilization as pointed out by Chidester (1937, 1939). These blocks were then aseptically taken out and exposed to vigorously growing cultures of *Auricularia auricula-Judae* in 1,000 c.c. Erlenmeyer flasks, each containing about 250 c.c. of 2.5% malt agar. These flasks, each containing 6 samples of wood, were kept at a constant temperature of 23°C. in darkness. Within a month, all the surfaces of the wood blocks were covered with smooth, white and felty mycelium but on the surface of the medium the mycelium formed a compact rugulose mat with shades of Light Vinaceous cinnamon to Vinaceous cinnamon mixed with white, raised and felty areas. In order to obtain a significant loss in weight the periods of exposure to fungal attack were 4 months and 8 months as recommended by Cartwright and Findlay (1946). After completion of the tests the flasks were opened, the superficial mycelium was carefully removed without damaging the wood blocks, weighed and oven dried in a similar manner, the resulting weights compared with the original and the percentage loss on dry weight of the sound wood was calculated (Pl. XXX, figs. 18, 19). The results are shown in Table 3.

TABLE 3

t-test of comparison of loss per cent in dry weight of wood blocks of elder exposed to *Auricularia auricula-Judae* after 4 and 8 months

Months	Number of replicates	Mean loss in dry wt. (%)	S.E.	<i>t</i>	Probability
4	12	12.3	± 0.89	8.65	< .01
8	12	25.4	± 1.23		< .01

Table 3 shows that the average losses in weight of elder wood in cultures, due to *Auricularia auricula-Judae*, are 12.3 ± 0.89 and 25.4 ± 1.23 after 4 and 8 months respectively. When the respective values of the loss in weight in 4 and 8 months are compared, the loss in weight after 8 months is nearly twice of that after 4 months. The loss is highly significant with a $t = 8.65$, for 22 degrees of freedom. The test-pieces have lost a high percentage of their original weight and, therefore, the wood has low resistance to decay. Findlay (1938) has classified the timber which he tested into five groups in response to their natural resistance to decay and accordingly elder wood falls under his 'non-resistant' group as this class undergoes average losses 10–30% during 4 months' test.

The moisture-contents of the wood blocks at the commencement of the experiment and 4 and 8 months later were determined. The average moisture-content of the wood blocks before they were exposed to fungal action was 98.3% while that after 4 and 8 months' tests were 94% and 83.4% respectively. No attempt was made to control the exact moisture-content during the experiment, but it was necessary only to find out whether the moisture-content was above the fibre-saturation-point or not for the activity of the wood-rotting fungus in bringing about the loss as pointed out by Cartwright and Findlay (1945).

INOCULATION EXPERIMENTS

Inoculation experiments were carried out with cut twigs in the laboratory and also with living trees in the field. In the former case the method followed by Brooks and Moore (1923) was followed. In this experiment young twigs of *S. nigra*, about 4" long, were kept with their ends in water. Their upper ends were cut off and spore-suspensions of *Auricularia auricula-Judae* in sterile water were applied to the freshly exposed surface and never allowed to dry out. These were then placed under a moist bell-jar at 23°C. in darkness. Longitudinal sections were made through the inoculated ends of the twigs at intervals of 24, 48, 72 and 96 hours after the spores had been added. Sections cut after 48 hours show that the majority of the spores had germinated on the surface and had sent hyphae down into the elements of the wood ultimately forming a vigorous mycelium in about 96 hours. Since these twigs could not be kept in an actively growing condition, their invasion by *Auricularia auricula-Judae* could not be regarded as an evidence for its parasitic action on trees. Nevertheless, it can be said that, if by chance the spores somehow alight on the surface of the wound, they can germinate on the surface under suitable conditions producing germ-tubes which in some way pass down into the elements of the wood.

Field inoculations were started on healthy living trees of *Sambucus nigra* and *Sambucus racemosa* in April, 1950. The trees inoculated were either young, about 2 to 3 years old or much older and were growing in a well-lighted open situation. In the latter case only the lower branches were inoculated. Inoculations were made at different times of the year for a period of ten months (March, 1950 to January, 1951). The operation involved was to make a cross incision through the bark and the branches were inoculated by transferring actively growing mycelium into the wound by carefully lifting a flap of the bark still attached to it. In case of a very young twig longitudinal triangular incision was first made upwards through the outer skin in order to obtain a flap of the skin still attached to it. The inoculum was then placed into the wound under the flap. Before inoculation the surface of the twig was wiped out with absolute alcohol and subsequently washed with sterile distilled water in order to free the surface from contamination as far as practicable. Each flap was then covered with moist absorbent sterile cotton-wool with a piece of thick paper over it to prevent drying out and firmly bound with a string. Two types of inocula were used for this experiment and these were taken from monosporous and dicaryotic mycelia made available for the purpose. At the beginning, twelve inoculations with monosporous and dicaryotic mycelia were made, six of each, keeping adequate controls. In case of *Sambucus racemosa* only three inoculations with each type of mycelium were done on the skin with controls below. The string and the cotton-wool were removed after a month and the inoculated branches were left exposed in that condition. At this stage no external sign of infection was, however, visible.

After about four months the trees were again examined and it was found that, in all cases, both *S. nigra* and *S. racemosa* seemed to have taken up the infection (Pl. XXIX, figs. 11, 12). The wounds were gaping open and somewhat swollen only in case of *S. nigra*. The invaded wood was discoloured and somewhat dark brown in colour. The controls remained flat and there was no such discolouration of the wood below the bark. After about 8 months the infected wounds became more conspicuous and there was considerable amount of swelling around the wounds in case of *S. nigra* but no such swelling was observed in *S. racemosa*. At this time no fruiting bodies of the fungus were formed and there was no other indication of decay. This was only to be expected owing to the very slow growth of the fungus even when grown on culture media under favourable conditions. As there were no other external effects of inoculation the internal conditions of the infected branches were examined microscopically. Mycelium was found within the tissues

of all the branches inoculated. It was, however, not uniformly distributed within the tissues of the host. In both *S. nigra* and *S. racemosa*, the hyphae were present in the bark and living cortex but especially abundant in the peripheral portion of the wood. The pith and inner layers of the wood were without any trace of hyphae. In the cortex, the hyphae ramified through the intercellular spaces but were also found penetrating the living cells. They spread more rapidly through the vessels in a longitudinal direction than in the cortex. Penetration in the transverse direction was rather slow. Very little can be said at present as to the ultimate effects produced by the advanced mycelium and the greatest distance to which the mycelium had penetrated into the wood from the point of infection. It may, however, be said that in case of *S. nigra* the hyphae traversed about half an inch in 4 months and to about an inch in 8 months from the point of infection in longitudinal direction while in *S. racemosa* this was much less being 8 mm. in about 4 months. All these point to the fact that *Auricularia auricula-Judae* under conditions favourable to infection is able to infect elder trees. This was further confirmed by re-isolating the fungus from different infected lesions.

Another set of experiments was started in October, 1950, when young twigs of *S. nigra* were inoculated with dicaryotic mycelia. After a period of 4 months these were examined and positive results were also obtained. On the whole, no difference was noted between inoculations with monosporous and dicaryotic mycelia.

DISCUSSION

A study on *Auricularia auricula-Judae* and its effects in the living trees and on sterilized wood blocks make it possible to discuss in general some of the salient points regarding the relations that exist between the fungus and the host. Fructifications of the fungus are commonly found to grow on the trunk and older branches of living elder trees (*Sambucus nigra*) and, as such, the fungus is often supposed to be a parasite. At present, there exists some difference of opinion among the investigators regarding the definition of a true parasitic wood-rotting fungus. According to some, parasites are those fungi which grow upon living hosts while others regard those as parasites which actually derive nourishment from the living tissues of such hosts. Therefore, in order to determine whether a wood-rotting fungus is strictly parasitic or not, it is essential to find out the presence of living elements within the tissues invaded and whether the fungus actually attacks and destroys such cells. Microscopic examinations of the different regions of elder wood shows that the only living elements in the sapwood are the parenchyma cells filled with starch grains while the fibres and the cells constituting the vessels have already died. In the heartwood, on the other hand, the elements are all dead and the food materials have been transferred out of the parenchyma cells during the process of maturation. It is, therefore, not imperative that a fungus attacking the sapwood would necessarily invade the living parenchyma cells, since the ultimate death and disintegration of such cells may be incidental during the process of digestion of the dead elements of the wood by the activity of the fungus.

Inoculation experiments on healthy elder trees (*S. nigra* and *S. racemosa*) with *Auricularia auricula-Judae* carried out in the field have shown that the fungus can invade living tissues of the cortex through wounds in the bark and the cambium is killed at the place of infection. The mycelium is both inter- and intracellular. In such cases the progress of decay would be from the bark to the woody portion of the stem. The course of infection, however, depends entirely on the initial point of infection, whether on the injured bark or on wounds due to cutting down of the branches. It has also been experimentally proved that under controlled conditions the spores of *Auricularia auricula-Judae* are able to germinate on freshly cut surfaces of elder twigs and can send down hyphae into the tissues of the host. In such a case, it is apparent that in all probability the woody portion of the stem would be

attacked first and the casual agent after entering the host would spread from the centre to the periphery of the stem. It has not been possible to study the progress of such an infection in the field. From the results of inoculation experiments, however, it can be regarded as a wound parasite killing the living cells of normal vigorous trees although the fungus shows a preference to grow in the wood deriving its food entirely from the dead wood elements and in extreme cases cause severe heart rots.

Whenever fructifications of *Auricularia auricula-Judae* were collected for this investigation, in most cases it was found to grow upon the stub of a broken branch or the old trunk of a living tree which was almost in the process of dying. In such cases, both sapwood and heartwood were particularly decayed. In some cases the sporophores were, however, seen to burst through the bark of living older branches borne on the decaying trunk and this may be regarded as a positive case of parasitism. Microscopic examination of the branch revealed the presence of mycelium in the living tissues and in the sapwood from which cultures were made. The bark became loose, separated easily and a mass of whitish mycelium was found in between the bark and the sapwood.

During field study it has been observed that the trees at Hopetoun, which were growing in a shady, low and moist situation, were attacked by the fungus. Trees growing wild at other places on higher land have been found to be somewhat protected from infection possibly due to lower humidity of the air which is unfavourable for spore-germination. It can, therefore, be assumed that the fungus does not usually attack trees of normal vigour but it would spread if the trees were unfavourably situated. It has already been stated that the foresters and landowners regard elder as a plant of little value and as such its branches are usually heavily cut down at any time of the year forming open wounds. This also has been the fate of the infected trees at Hopetoun and as such *Auricularia auricula-Judae* being a wound parasite possibly entered through these wounds and brought about infection. The fungus is common there as a saprophyte on dead branches and during November to February forms fructifications. The spores, after being shed and carried away by the wind, alight on the cut surfaces of the branches or on wounds in the bark and bring about infection under favourable conditions prevailing there.

The amount of damage in the field caused by *Auricularia auricula-Judae* has not, however, been determined. Experiments on durability tests on elder samples carried out in the laboratory have shown that elder wood is 'non-resistant' to decay due to *Auricularia auricula-Judae*, since this class suffers average losses in weight from 10-30% during 4 months' test. There is no doubt that this loss is quite significant and such injuries would cause serious deterioration in the quality of the wood.

SUMMARY

1. *Auricularia auricula-Judae*, because of its wide range of distribution in the temperate and tropical regions throughout the world and its adaptability to grow on a great variety of wood, is undoubtedly responsible for the deterioration of large quantities of wood annually. Although commonly found as a saprophyte, it has been found to grow as a parasite on elder (*Sambucus nigra*).

2. The fructifications of the fungus have been thoroughly described and their peculiarities noted. They are readily recognized in the field by their soft, gelatinous, semi-transparent, irregularly lobed, sometimes conchiform or auriform and sessile fruiting bodies, becoming hard and horny on drying. The fruit-body is composed of hyphae showing numerous clamp-connections.

3. Inoculation experiments on healthy standing trees of both *S. nigra* and *S. racemosa* carried out in the field have shown that the fungus, in both cases, can attack the living tissues through wounds in the bark but in all cases the growth of the mycelium has been very slow. The fungus, however, shows a preference to grow in the wood and in extreme cases causes severe heart rots. It has also been experimentally proved that under laboratory conditions the spores of *Auricularia auricula-Judae* are able to germinate on freshly cut surfaces of young elder twigs and can send down hyphae into the tissues of the host. From the results of inoculation it can be regarded as a wound parasite on normal vigorous trees.

4. For this investigation fructifications were found growing upon living trunks and comparatively older branches of elder, usually bursting open through the decayed or cracked bark. Close examination showed that the younger branches had been regularly cut down annually whereby open wounds were exposed. Through these wounds the fungus presumably had infected the trees and ultimately attacked the heartwood, thereby rendering it unfit for commercial use. These trees, which were growing on a low, moist situation, were found to be attacked with the fungus. Most species at other places growing on higher land were found to be protected from infection, possibly owing to lower humidity of the air and the consequent poorer condition for spore-germination.

5. In the early stage of attack the fungus produces incipient rot pockets at the periphery of the wood but in an advanced stage of decay both sapwood and heartwood are attacked which are rendered light, soft and spongy, easily breaking up into sections. The heartwood becomes hollow at the centre and the rotten wood eventually falls into small flakes. Thin sheets of conspicuous whitish mycelium are found in the shrinkage cracks along the grains of the wood. The distribution of hyphae within the decayed wood is fairly uniform, being particularly abundant in the medullary rays, and to a certain extent in the vessels in the early stages of decay. The clamp-connections are more frequent in the wider hyphae. Bore-holes are numerous in the advanced stage of decay and the fibres and vessels are often completely filled up with mycelial warts associated with yellowish gum-like material.

6. Chemical changes in the wood during the process of decay have been tested with micro-chemical stains in common use. In the partially decayed wood the change consists primarily in gradual delignification of the highly lignified parts of the secondary walls of the elements and, as such, the residual cellulosic materials show more intense staining reaction.

7. Experiments on durability tests in the laboratory have shown that elder wood is non-resistant to decay due to *Auricularia auricula-Judae*, since this class suffers average losses in weight from 10-30% during 4 months' test. The average losses in weight of elder wood in cultures have been 12.3% and 25.4% after 4 and 8 months respectively.

8. Cultures of the fungus have been made from spores and tissues of the decayed wood. Their characteristics have been followed on potato-dextrose agar and malt agar. In all isolations the cultures started in the same way and the average rate of growth on both the media has been slow and more or less the same, but a little faster on malt agar. It is a white rot fungus as it gives positive oxidase reactions on Bavendamm's medium.

9. Under ordinary conditions of light and temperature of the laboratory, typical fructifications of *Auricularia auricula-Judae* bearing normal basidia and viable basidiospores have been obtained in artificial cultures originating from the infected tissues of the elder. Various devices adopted to induce the cultures to fructify have been described. Cultures on various agar media, however, have not fructified at all.

10. The basidiospores have germinated readily in water and on agar media kept under various conditions of light and temperature. In water at 18°C. they have germinated only by producing conidia but at 23°C. many of the spores have given rise to much-branched germ-tubes bearing clusters of conidia at the tips of short lateral branches. On agar media under all conditions, direct germination by producing germ-tubes has been observed.

ACKNOWLEDGEMENTS

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EXPLANATION OF PLATES

Plate XXVIII

- FIG. 1. A group of fructifications of *Auricularia auricula-Judae* on a dead trunk of elder (*S. nigra*).
- „ 2. A group of young fruit-bodies of the fungus on the cut end of a stout branch the lower portion of which bore living shoots.
- FIGS. 3 and 4. Cross-sections of the infected wood showing various stages of decay.
- FIG. 5. Longitudinal section of the wood showing the rot; the pith has been completely destroyed.
- „ 6. Mycelial sheets in between the shrinkage cracks formed along the grains of the wood.

Plate XXIX

- FIGS. 7-9. Fructifications of *A. auricula-Judae* formed at the edge of the Petri dishes in artificial culture.
- FIG. 10. Irregular fructifications formed at the rim of the test-tube within the flask.
- „ 11. Inoculated twigs of *S. nigra* showing the infection (right) and the control (left) after 4 months.
- „ 12. Inoculated twigs of *S. racemosa* showing infections after 4 months.

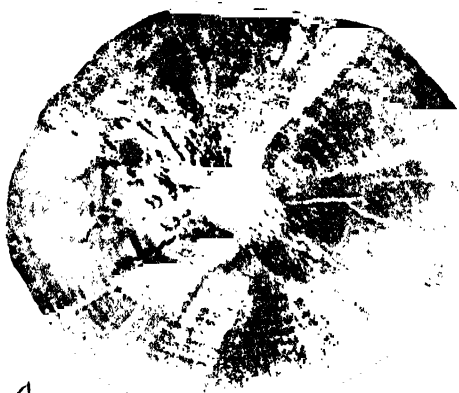
Plate XXX

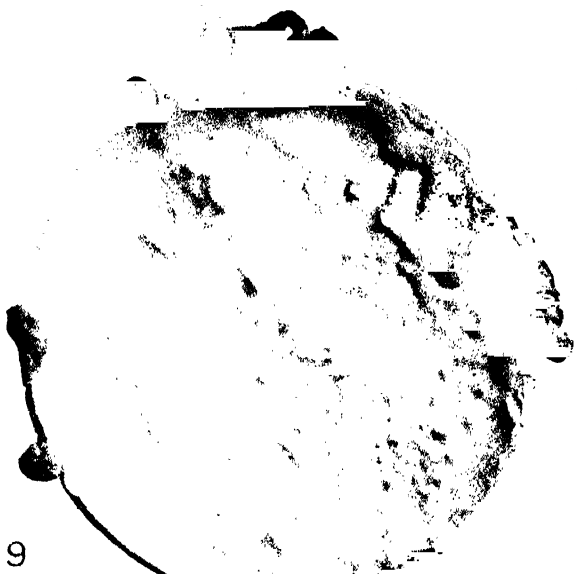
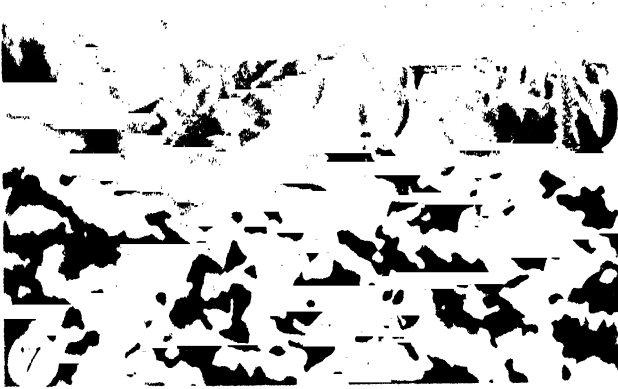
- FIG. 13. 21-days-old culture of *A. auricula-judae* on malt agar at 23°C. in darkness.
 „ 14. Cultures of the fungus, 21 days old, on potato-dextrose agar and malt agar in darkness at 18°C. (a, c) and 23°C. (b, d) respectively.
 „ 15. Spores of *A. auricula-judae* germinating directly into mycelia on malt agar ($\times 200$).
 FIGS. 16 and 17. Germinating spores producing conidia ($\times 800$).
 FIG. 18. Wood block cultures of the fungus, 30 days old, for testing decay resistance.
 „ 19. Wood blocks of elder showing decay after 8 months' exposure to *A. auricula-judae*.

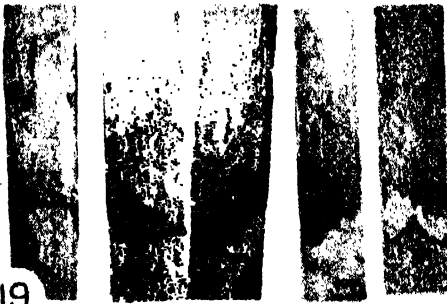
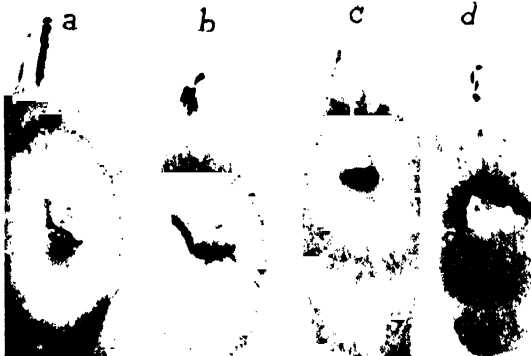
Plate XXXI

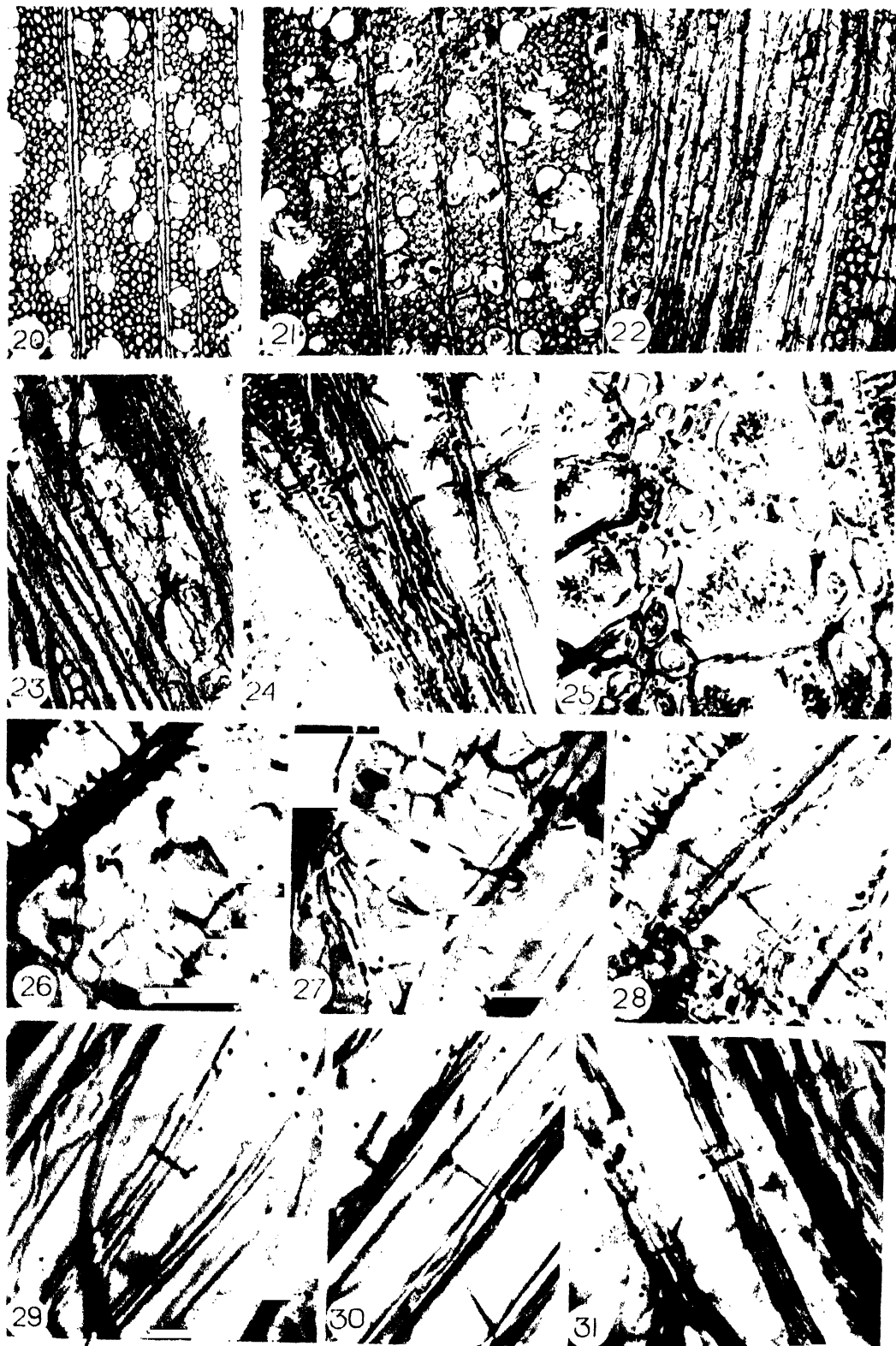
- FIG. 20. Part of a transverse section of normal elder wood ($\times 50$).
 „ 21. Part of a transverse section of a partly decayed elder wood; masses of hyphae are visible within the vessels ($\times 50$).
 „ 22. A general view of partly decayed elder wood in cross-section showing distribution of the hyphae and spiral shrinkage cracks within the fibres ($\times 50$).
 „ 23. A general view of the wood in transverse section in an advanced stage of decay showing masses of hyphae in the vessel, the bore-holes and spiral shrinkage cracks in the fibres ($\times 50$).
 „ 24. A vessel with hyphae penetrating through the bordered pits ($\times 190$).
 „ 25. A view of the cross-section of a partly decayed elder wood showing abundant hyphae within the wood elements ($\times 225$).
 „ 26. Wider hyphae with clamp-connections passing through the pits ($\times 450$).
 „ 27. A hypha penetrating the bordered pits and also the border part of a pit ($\times 450$).
 „ 28. A hypha passing through the pits of the medullary ray cells ($\times 450$).
 FIGS. 29-31. Completed penetration of the cell-walls by the hyphae (Figs. 29, 31) after their emergence from the bore-hole. The apex of a larger hyphae has become attenuated into a relatively fine point of considerably smaller diameter; it has reached the middle lamella and is visible within the bore-hole (Fig. 30) ($\times 450$).

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DEVELOPMENT OF THE SKULL IN CATFISHES

PART I. DEVELOPMENT OF CHONDROCRANIUM IN *Silonia*, *Pangasius* AND *Ailia* (SCHILBEIDAE)

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INTRODUCTION

Siluroids form a large bulk of the order Ostariophysi and have undergone a number of interesting modifications in various directions. Very little work has been done on the development of skull in Indian catfishes. Bhimachar (1933) described the morphology of adult skulls of certain Indian catfishes. Karandikar and Masurekar (1954) have given an account of the Weberian ossicles and other related structures of *Arius*. Kumar (1955) published a short note on the skull of *Eutropichthys*. But no work has been done on the development of chondrocranium of any Indian catfish.

McMurrich (1884), Wright (1885) and Ryder (1887) have given incomplete descriptions of the cranium of adult *Amiurus*, the skull of *Hypophthalmus* and the chondrocranium of *Ictalurus* respectively. Later, Kindred (1919) described the development of skull in the same fish *Amiurus*. Others like Harry (1953) and George (1954) have added to our knowledge of African catfishes and of *Clarius* respectively.

On the suggestion of Dr. L. S. Ramaswami, I have taken up the study of catfishes of India with a view to see if the cranial osteology would help in the systematics of the group and the present paper on the development of chondrocranium of *Silonia silondia*, *Pangasius pangasius* and *Ailia coila* of the family Schilbeidae forms the first part of my study.

MATERIAL AND METHODS

The developing fry of *S. silondia*, *P. pangasius* and *A. coila* were collected from Mahanadi river (India) and were fixed both in Bouin's fluid and in 4% formalin. The sections were cut at 10 micra thick and stained in iron haematoxylin, Delafield's haematoxylin, haemalum and Mallory's triple. The latter gave very good results. The following stages of each member have been studied.

	Head-length, mm.	Body-length, mm.	Remarks
<i>Silonia silondia</i> Ham.:			
Stage 1	2.0	8.0	Wax model
Stage 2	2.5	11.0	Transverse sections
Stage 3	3.0	16.0	T.S.
Stage 4	3.5	18.0	Wax model
Stage 5	4.5	23.0	T.S.
<i>Pangasius pangasius</i> Ham.:			
Stage 1	2.5	13.0	T.S.
Stage 2	3.0	17.0	Wax model
<i>Ailia coila</i> Ham.:			
Stage 1	2.5	13.0	T.S.
Stage 2	3.0	16.0	Wax model

Wax model reconstructions of some stages have been made to study the chondrocranium. In addition, van Wijhe's (1922) technique was adopted in some of the stages for the sake of comparison.

OBSERVATIONS

Stage 1. The chondrocranium of *Silonia silondia*
(8 mm. stage; head-length 2 mm.; Figs. 1 and 2)

This is the earliest stage in which the chondrocranium has been studied. The chondrocranium at this stage is elongated and differs very much from the fully formed chondrocranium.

Ethmoid region.—The ethmoid plate (Figs. 1, 2, EP) (*trabecula communis*), formed by the fusion of the trabeculae, is in the form of flat plate, the ends of which are slightly depressed in the region of the nasal sacs. The ethmoid plate does not possess lateral extensions forming a complete floor (*solum nasi*, Gaupp, 1906) for the olfactory sacs. Anteriorly the ethmoid plate rises in the form of a nasal septum which is slightly broad and does not extend posteriorly. The *lamina precerebralis* (LPC) or the so-called nasal septum forms the anterior boundary for the cranial cavity. The anterior part of the ethmoid plate does not extend in the form of ethmoid cornua which are quite prominent in the American catfish *Amiurus* (Kindred, 1919). The ventral surface of the ethmoid plate is slightly concave. The lamina precerebralis is connected with the orbital cartilages by means of a sphenoseptal commissure (SSC). The olfactory foramen (Fig. 2, AOF) is large and bounded in front by the lateral edge of the lamina precerebralis, behind by *lamina orbitonasalis* (LON), dorsally by the sphenoseptal commissure and ventrally by the ethmoid plate. A thin lamina orbitonasalis is developed connecting each orbital cartilage with the ethmoid plate and possesses a cup-like socket ventro-laterally for articulation of the pterygoid process. The ethmoid plate continues posteriorly as the trabeculae (Figs. 1, 2, TC) and unites with the parachordal plate (Fig. 1, PC).

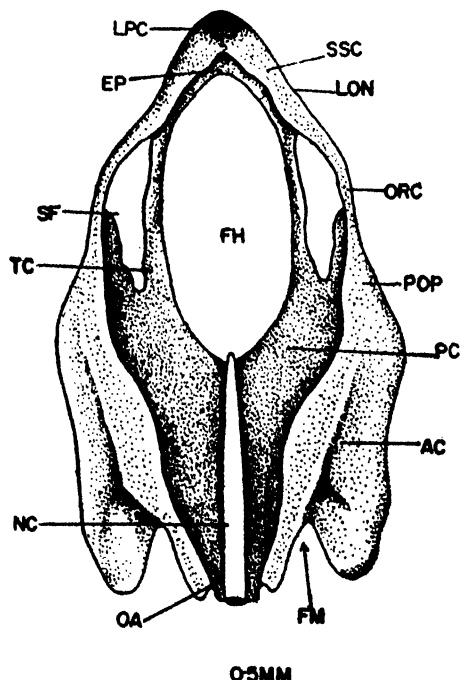


Fig. 1. Dorsal view of the chondrocranium of *Silonia silondia*, 8 mm. stage (wax model).

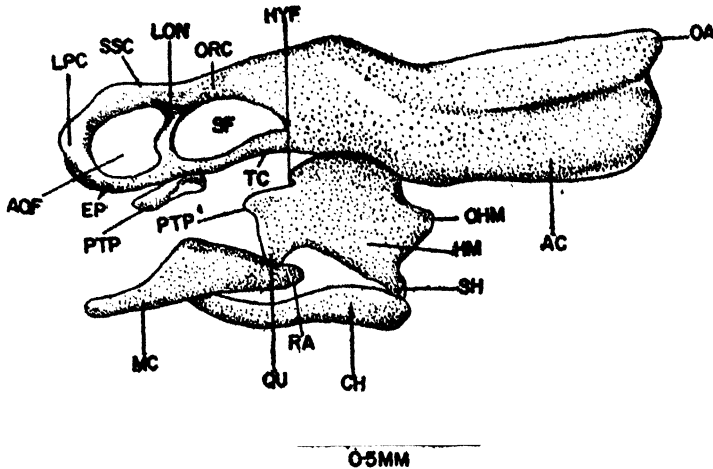


FIG. 2. Lateral view of the chondrocranium of *Silonia silondia*, 8 mm. stage.

Orbito-temporal region.—The lateral wall in the orbital region is formed by the trabeculae ventrally and the orbital cartilages (Figs. 1, 2, ORC) dorsally. The two orbital cartilages are in the form of long plate-like structures slightly curved in the middle orbital region and join the sphenoseptal commissure in the ethmoid region. Posteriorly the orbital cartilages (alisphenoid of Kindred, 1919) are laterally compressed at the region where they unite with the auditory capsule (AC). The orbital cartilages are not connected by an epiphysial bar as noticed in 10 mm. stage of *Amiurus* (Kindred, 1919).

The trabeculae which are seen extending from the antero-lateral corners of the basal plate are in the form of rod-like cartilages and enclose a large hypophysial fenestra (Fig. 1, FH). Anteriorly the trabeculae are united to form trabecula communis or the ethmoid plate.

On each side in front of the basal plate, between the trabecula and orbital cartilage, is a large foramen through which the optic, oculomotor, trigeminal, abducens and facial nerves pass from the cranial cavity. This fontanelle—the sphenoid fissure (Figs. 1, 2, SF)—extends nearly the whole length of the trabeculae in the chondrocranium.

There is no lateral commissure or the pila antotica developed in this stage of the chondrocranium.

Auditory region.—The auditory capsule at this stage is not completely formed except for a small wall in the anterior region of the capsule, and the cavity of the auditory capsule opens into the cranial cavity. The basicapsular fenestrae are absent. Anteriorly the cartilage of the auditory region is continuous with the orbital cartilages. The three septa, septum semicircularis anterior, laterale and posterius, separating the semicircular canals of the internal ear are noticed in the auditory capsule.

The hyomandibular cartilage (Fig. 2, HM) articulates at the ventro-lateral region of the auditory capsule below the bulge of the lateral semicircular canal. There is no projecting shelf from the auditory capsule in the region of articulation of the hyomandibula. The actual region of articulation of the hyomandibula is very small when compared with the extent to which the hyomandibula extends in the auditory region. The auditory capsule externally shows slight swellings for the accommodation of the anterior, lateral and posterior semicircular canals.

Basal plate and notochord.—The basal plate is in the form of a shallow bowl in the centre of which passes the notochord. As stated above, the parachordals are fused with the auditory capsules, and thus the basicapsular fenestrae are eliminated.

The auditory capsules are not joined by a tectum synoticum as seen in other teleosts. Posterior to the otic capsules the occipital arches are seen, and there is no pronounced demarcation between them. The glossopharyngeal and vagus nerves pass through a fissure—the fissura metotica (Fig. 1, FM)—in the posterior region between the auditory capsule and the occipital arch.

The notochord passes in the middle region of the basal plate, and it is intracranial in position throughout its length in the basal plate. Anteriorly the notochord projects into the hypophysial fenestra, and there is no separate basi-cranial fenestra noticed as in other teleosts. The notochord is triangular in cross-section.

Occipital region.—The occipital region is continuous with the auditory capsules posteriorly and does not possess a roofing cartilage. The auditory capsules are fused with the occipital arch in front of the fissura metotica.

The visceral arches.—The different elements of the mandibular, hyoid and branchial arches could be made out in this stage. The first visceral arch, as in other teleosts, divides into the upper palatoquadrate bar and a lower Meckel's cartilage forming the lower jaw. The palatoquadrate bar has a pterygoid process chondrifying independently. It is a slender slightly curved bar of cartilage lying on either side of the ethmoid plate. The pterygoid process (Fig. 2, PTP) articulates lateral to the ethmoid plate in a cup-like depression in the region of the lamina orbitonasalis. It is a cylindrical rod slightly swollen in the middle region and is posteriorly attached to the trabecula by means of muscle fibres. The quadrate (QU) portion of the pterygoquadrate bar is separated from the pterygoid process and is fused with the hyomandibula. There is a small anterior projection—the processus pterygoideus of the quadrate (PTP')—extending from the quadrate and is unconnected with the anterior portion. The symplectic part of the hyosymplectic is not seen in the stage described as is also the case in most of the siluroids studied.

Meckel's cartilages meet anteriorly and do not fuse, but are connected by connective tissue. Posteriorly Meckel's cartilage articulates with the quadrate. There is a small retroarticular (Fig. 2, RA) process of Meckel's cartilage extending posterior to the region of articulation.

In the second visceral arch, the hyomandibula is in the form of a plate and articulates with the chondrocranium in the region of lateral semicircular canal of the auditory capsule. The plate-like hyomandibula possesses a posterior extension and antero-ventrally it is fused with the quadrate as noted above. The hyomandibular branch of the facial nerve passes in a niche (Fig. 2, HYF) in front of the hyomandibula. The hyomandibula is connected ventrally with the ceratohyal by a small interhyal (Fig. 2, SH) which is seen as a part of the hyomandibula.

The hypohyals, which are not distinct from the posterior ceratohyals, are in the form of stout cartilages extending in front of the copula. The hypohyals have grooves for the passage of the hyoidean artery. All the five branchial arches are developed. The copula which is virtually formed by the fusion of the basibranchials is a continuous rod and on either side of it at intervals are noticed the ceratobranchs. In connection with the first two branchial arches the epibranchs and pharyngo-branches are seen.

Stage 2. The chondrocranium of *Silonia silondia* (11 mm. stage; head-length 2.5 mm.)

In this stage there is a general growth of all cartilages. The solum nasi of Gaupp (1906) has just developed forming the floor on the posterior region of the nasal capsule. In the orbito-temporal region an incomplete epiphysial bar is seen between the orbital cartilages.

The notochord does not extend into the hypophysial fenestra as observed in the earlier stage. A tectum synoticum connecting the two auditory capsules is

noticed, and the tectum is not distinct from the tectum posterius of the occipital arch.

In the visceral arch skeleton the processus pterygoideus of the quadrate has grown forwards, but remains separate from the anterior portion of the pterygoid process.

Stage 3. The fully formed chondrocranium of *Silonia silondia*
(18 mm. stage; head-length 3.5 mm.; Fig. 3)

1. *Neurocranium*.—The chondrocranium at this stage is fully formed and has grown greatly, and appears to be more bulky and wider than in the earlier stages described.

Occipital region.—The occipital arches are now well developed and are connected dorsally by a thin roofing cartilage, the tectum posterius (TP), which is fused with the tectum synoticum of the auditory capsules anteriorly, as stated above. The glossopharyngeal and vagus nerves pass through a common exit which is in the form of a wide fissure—the fissura metotica—between the auditory capsule and the occipital arch.

Auditory region.—Each auditory capsule shows externally enlargements for the semicircular canals (PASC, PLSC, PPSC). In the anterior part of the auditory capsule where it is continuous with the orbital region is a foramen for the exit of the otic branch of the facial nerve innervating the sensory canal. There are medial walls in the anterior and posterior regions of the auditory capsules separating the cavum labyrinthii from the cavum cranii, and in the middle region the cavity of the auditory capsule opens freely into the cranial cavity. All the three septa are completely developed. The septum semicircularis anterior is a thin bar of cartilage extending from the anterior wall of the auditory capsule to the mid-ventral surface of the roof parallel to the long axis of the body. The septum semicircularis laterale is found at right angle to the septum semicircularis anterior and extends posteriorly very near the posterior septum. The septum semicircularis posterior is situated in the same plane as the anterior septum and continues as the medial wall to the posterior end of the auditory capsule.

The hyomandibular cartilage articulates at the region of the lateral semicircular canal of the auditory capsule and at this region the auditory capsule projects in the form of a shelf over the hyomandibula. The auditory capsules are connected by means of tecti (Fig. 3, TSY, TP) as in the 11 mm. stage.

Basal plate and notochord.—The basal plate (Fig. 3, BP) is extensive and its lateral edges are fused with the auditory capsules. The parachordals are united in the middle line in front of the notochord. There are no basicapsular fenestrae and commissures as in *Amiurus* (Kindred, 1919). At the level of the lateral semicircular canals on the dorsal surface of the basal plate is a pair of deep grooves extending backwards to the posterior end of the cranium. These grooves lodge the sacculus and lagena of the internal ear and they communicate with each other across the anterior ends of the grooves by a transverse canal—the sinus impar which projects backwards into the cavum sinus impar is immediately above the notochord. Posteriorly the sacculus is completely covered over by a cartilaginous cup.

The notochord is covered with cartilage both dorsally and ventrally and does not extend into a basiscranial or hypophysial fenestra as seen in the earlier stage (8 mm.). Anteriorly the notochord is narrow and becomes broad in diameter at the posterior end of the cranium.

Orbito-temporal region.—The orbital cartilages (Fig. 3, ORC) are in the form of large vertical plate-like cartilages extending from the lamina orbitonasalis to the anterior end of the auditory capsule as also seen in the earlier stage. These cartilages have grown considerably since the earlier stage, and in the anterior region

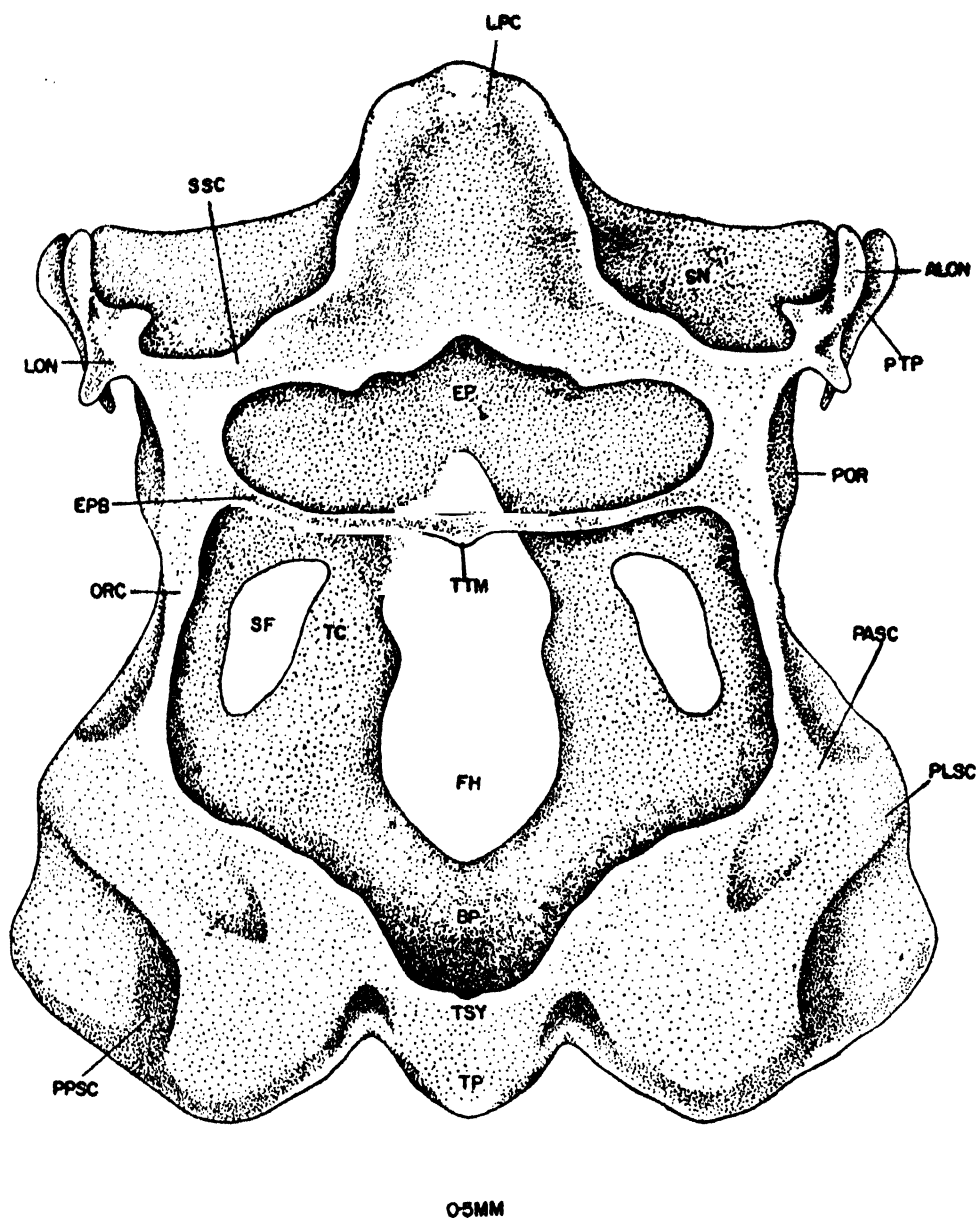


FIG. 3. Dorsal view of the fully formed chondrocranium of *Silonia silondia*, 18 mm. stage (wax model).

they are connected by an epiphysial bar (EPB) which is almost complete, thus dividing the large dorsal fontanelle into anterior and posterior ones.

The trabeculae (Fig. 3, TC) are now plate-like anteriorly fusing with the orbital cartilages in front of the sphenoid fissure (Fig. 3, SF) forming a vertical plate—the preoptic root of the orbital cartilage shielding the side of the anterior part of the brain case. The hypophysial fenestra (FH) at this stage is much reduced on account of the widening of the trabecular plate. There is no lateral commissure or pila antotica noticed unlike other teleosts.

Ethmoid region.—The ethmoid region of the fully formed chondrocranium differs very much from the other stages studied. The solum nasi is greatly developed and forms the floor for the nasal sacs. The lamina precerebralis is enlarged and does not extend posteriorly. A typical nasal septum as seen in other teleosts is not developed. The olfactory sacs now possess a floor formed by the solum nasi, whose side wall and posterior boundary are formed by the extension of the lamina orbitonasalis. The lamina precerebralis extends posteriorly as a process forming a roof for the anterior part of the olfactory peduncles. The olfactory foramen is very much reduced when compared with the earlier stage. The foramen olfactorium evehens and advehens are confluent, and the foramen is enclosed by the lamina precerebralis dorsally and ventrally by the ethmoid plate.

The ethmoid plate (Fig. 3, EP) or the trabecula communis is flat and forms the floor for the olfactory peduncles. The sphenoseptal commissure (SSC) is very much reduced as the lamina precerebralis extends backwards near the lamina orbitonasalis. The lamina orbitonasalis is developed extensively and connects the ethmoid plate with the orbital cartilage. Dorsally the lamina orbitonasalis is pierced by a foramen for the exit of the superficialis branch of the trigeminal nerve. The foramen orbitonasale is now situated more posteriorly indicating that the anterior part of the chondrocranium is elongated.

The olfactory sacs project posteriorly into a cavity formed in the lamina orbitonasalis so much so the olfactory sacs at this region possess a complete cartilaginous covering. The olfactory lobes lie on saucer-like lateral extensions of the ethmoid plate, the solum nasi, and are covered for the most part on the dorsal surface by a membrane.

2. **Visceral arches.**—In the upper pterygoquadrate bar, the pterygoid process (Fig. 3, PTP) which chondrified independently in the earlier stage is now developed into a thick rod, broad anteriorly and tapering posteriorly. The body of the quadrate is fused with the hyomandibula and possesses a narrow plate-like extension anteriorly representing the posterior portion of the pterygoid process or processus pterygoideus of the quadrate, and it is not continuous with the anterior portion. The anterior portion of the pterygoid process is attached on either side to the lamina orbitonasalis, and in the middle region the attachment is very intimate. The portion of the pterygoid process which is intimately attached to the lamina orbitonasalis is the reduced ethmo-palatine process; a rostro-palatine process of the pterygoid process seen in other teleosts is absent.

The hyomandibular cartilage is now completely formed and it is in the form of a large vertical plate articulating with the auditory capsule in the region of the lateral semicircular canal. The hyomandibula possesses a posterior narrow extension—the opercular process—and anteriorly has a niche for the passage of the hyomandibular branch of the facial nerve. It is also observed in the sections that, in the later stage (23 mm.), the nerve passes through the bone. Ventrally the quadrate is fused with the hyomandibula and does not possess a symplectic extension as seen in other teleosts. The ventral portion of the hyomandibula is connected with the ceratohyal by means of the small interhyal which appears to be fused with the ventro-posterior end of the hyomandibula.

Meckel's cartilages are separate from each other and possess large coronoid processes in front of their articulation with the quadrate. The retroarticular process of Meckel's cartilage is very much reduced.

The copula which is formed by the fusion of the basihyal with the basibranchials is a continuous rod, broad anteriorly and narrow posteriorly. The hypohyals are thick pieces of cartilages projecting in front of the copula. These cartilages bear a groove ventrally for the passage of the hyoidean artery. The hypohyal continues backwards as the ceratohyal which is expanded posteriorly and again becomes narrow at the region of attachment with the interhyal. The interhyal is a small piece of cartilage and is fused with the hyomandibula as stated above.

... All the five branchial arches are completely formed. The first three arches are attached on either side of the copula at regular intervals. But the fourth and fifth branchial arches appear to be free from the copula. The first four branchial arches possess hypo-, cerato-, epi- and pharyngobranchials, the fifth arch being represented by only the rod-like ceratobranchials. While pharyngobranchials of the first and second branchial arches are free, the pharyngobranchials of the third and fourth arches are fused with each other.

The chondrocranium of *Pangasius pangasius*
(17 mm. stage ; head-length 3.0 mm. ; Figs. 4, 5 and 6)

The chondrocranium of *Pangasius* is fully formed in a 17 mm. fry and it is more elongated than in *Silonia* previously described. The ethmoid region is very broad anteriorly and shows slight projections in the form of cornua on either side of the anterior end of the ethmoid plate. The lamina precerebralis (Figs. 4, 5, LPO) is narrower and extends considerably backwards thus reducing the olfactory foramen (Fig. 5, AOF). The solum nasi (Fig. 4, SN) forming the floor for the nasal sacs are much reduced and not as broad as in *Silonia*. The lamina orbitonasalis (LON) is thick and possesses an anterior extension (Fig. 5, ALON) which forms a sort of side wall for the posterior region of nasal sac.

The lamina orbitonasalis possesses dorsally a foramen (Fig. 5, FON) for the exit of the superficialis branch of the trigeminal nerve. The sphenoseptal commissure (SSC) connects the orbital cartilages with the lamina precerebralis. An internasal septum in a strict sense is not developed as in *Silonia*.

In the orbito-temporal region, the orbital cartilages are connected by an epiphysial bar (Figs. 4, 5, EPB) which divides the large fontanelle into anterior and posterior portions. Extending from the middle of the epiphysial bar there is a small taenia tectomedialis (Fig. 4, TTM) projecting into the posterior fontanelle. Anteriorly the orbital cartilage (Figs. 4, 5, ORC) is connected with the trabecula (TO) by a wide plate of cartilage—the preoptic root (Fig. 4, POR)—forming the side wall for the cranium at this region. Just behind the lamina orbitonasalis there is a preoptic fontanelle (POF) in the preoptic root of the orbital cartilage. The orbital cartilages are thinned out posteriorly and they are continuous with the postorbital processes of the auditory capsule (Fig. 5, AO). The hypophysial fenestra (Fig. 4, FH) is large and is bounded laterally by the broad plate-like trabeculae (Figs. 4, 5, TO). As in *Silonia* the optic, oculomotor, trigeminal, abducens and facial nerves pass through the wide fissure—the sphenoid fissure (SF)—which is bordered dorso-laterally by the extension of the orbital cartilage and ventrally by the trabecula. The sphenoid fissure extends from the region of the anterior part of the auditory capsule to the preoptic root of the orbital cartilage (POR). The internal carotid artery passes medially to the trabeculae in a typical way as noticed in other silurids. There is no trace of a myodome; the eye muscles are attached to the trabecular plate and do not penetrate into the cranial cavity.

The auditory region is similar in general plan to *Silonia*. The auditory capsule (Fig. 5, AO) is more elongated than broad. The dorsal posterior fontanelle extends far backwards and thus the tectum synoticum (Fig. 4, TSY) uniting the two auditory capsules is fused with the tectum posterius (TP) of the occipital region. Arising on each side of the antero-lateral corners behind the postorbital process (POP) of the auditory capsule is a foramen (Fig. 5, FOR) for the exit of the otic branch of the facial nerve which innervates the lateral line sensory canal. Enlargements for the accommodation of the semicircular canals could be noticed externally on the auditory capsules. The hyomandibular cartilage articulates with the auditory capsule in the region of the lateral semicircular canal. The auditory capsule does not project outwards in the region of articulation in the form of a shelf noticed in *Silonia*. The medial wall separating the cavity of the auditory capsule from the

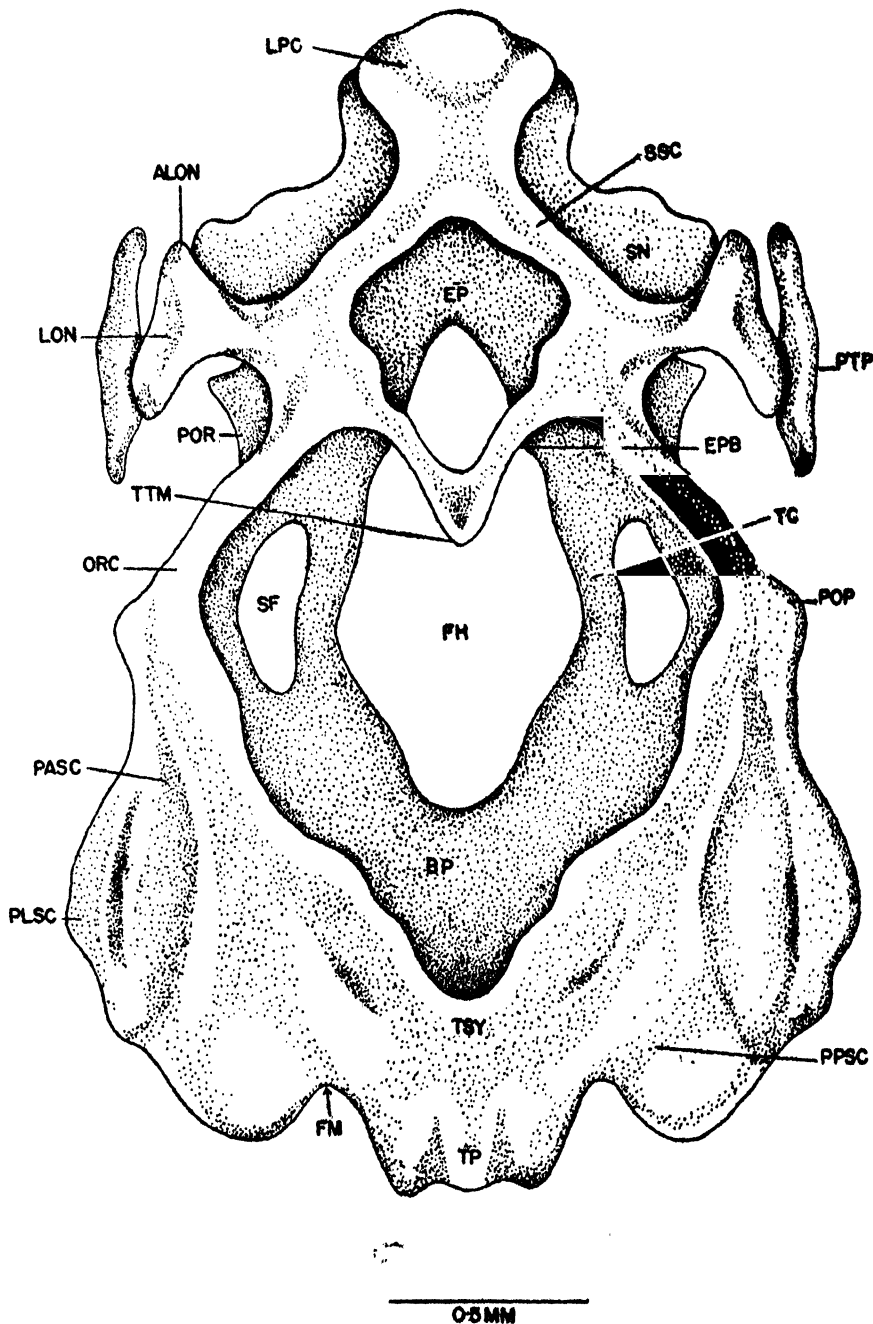


Fig. 4. Dorsal view of the fully formed chondrocranium of *Pangasius pangasius*, 17 mm. stage (wax model).

cavum cranii is confined to the anterior and posterior ends only and to a greater part the cavum labyrinthii opens into the cavum cranii.

The basal plate (Fig. 4, BP) is quite extensive and possesses a prominent basicapsular fenestra in the middle region of the floor and a large anterior basicapsular commissure. The plate extending behind the basicapsular fenestra can be recognized as basivestibular commissure as the glossopharyngeal nerve passes in

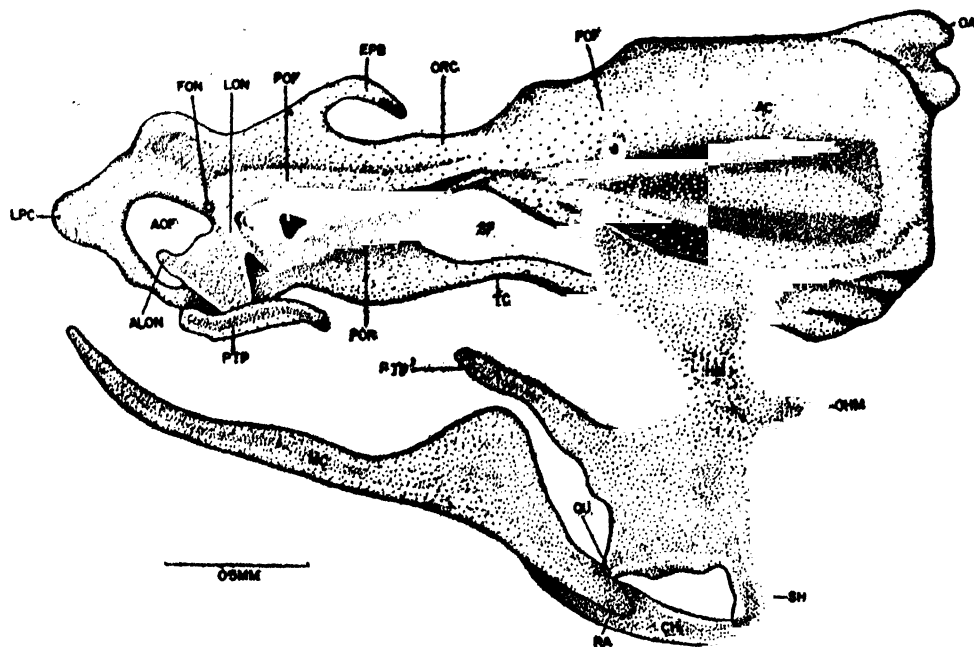


FIG. 5. Lateral view of the fully formed chondrocranium of *Pangasius pangasius*, 17 mm. stage.

the fissura metotica (FM) so also the vagus nerve. There is a pair of prominent recesses on the dorsal surface of the posterior region of basal plate for the accommodation of the sacculi of the internal ear as in *Silonia*. The paired nature of the parachordals is quite distinct in the posterior region, though to a greater length they are fused in the middle region.

The notochord which is slightly oblong in transverse section does not extend into the hypophysial fenestra. Anteriorly the notochord is completely enclosed in the basal plate, i.e. it is intracranial, while posteriorly it is more or less ventral to the basal plate.

The occipital arch (Fig. 5, OA) is continuous with the auditory region and possesses a tectum posterius fused with the tectum synoticum anteriorly as in *Silonia*.

Visceral arches.—In the first visceral arch the pterygoid process (Figs. 4, 5, PTP) which chondrifies independently extends on either side of the ethmoid region, and is intimately connected to the lamina orbitonasalis by connective tissue. The posterior part of the pterygoid process, which is continuous with the quadrate and hyomandibula, tapers anteriorly. The body of the quadrate is fused with the ventral part of the hyomandibula and articulates ventrally with Meckel's cartilage (Fig. 5, MC).

Meckel's cartilage on either side is a large cylindrical cartilage with a broad coronoid process in front of the region of articulation. The two Meckel's cartilages anteriorly meet but do not fuse in the middle line. The retroarticular (Fig. 5, RA) process is very much shortened in the fully formed chondrocranium almost ending next to the region of articulation with the quadrate (QU).

In the second visceral arch the hyomandibular cartilage (Fig. 5, HM) is a broad plate-like, hanging downwards from the region of the auditory capsule and possesses a gap in front for the passage of the ramus hyomandibularis facialis as in *Silonia*. Posteriorly in line with the above gap is a long projecting opercular process (OHM) in the form of rod-like cartilage. The hyomandibula continues postero-ventrally

as the stylohyal (SH) which connects the ceratohyal (CH) anteriorly. In the hyoid cornu the hypohyal (Fig. 6, HYH) continues as the ceratohyal which becomes broad in the posterior region and is connected with the hyomandibula by a stylohyal.

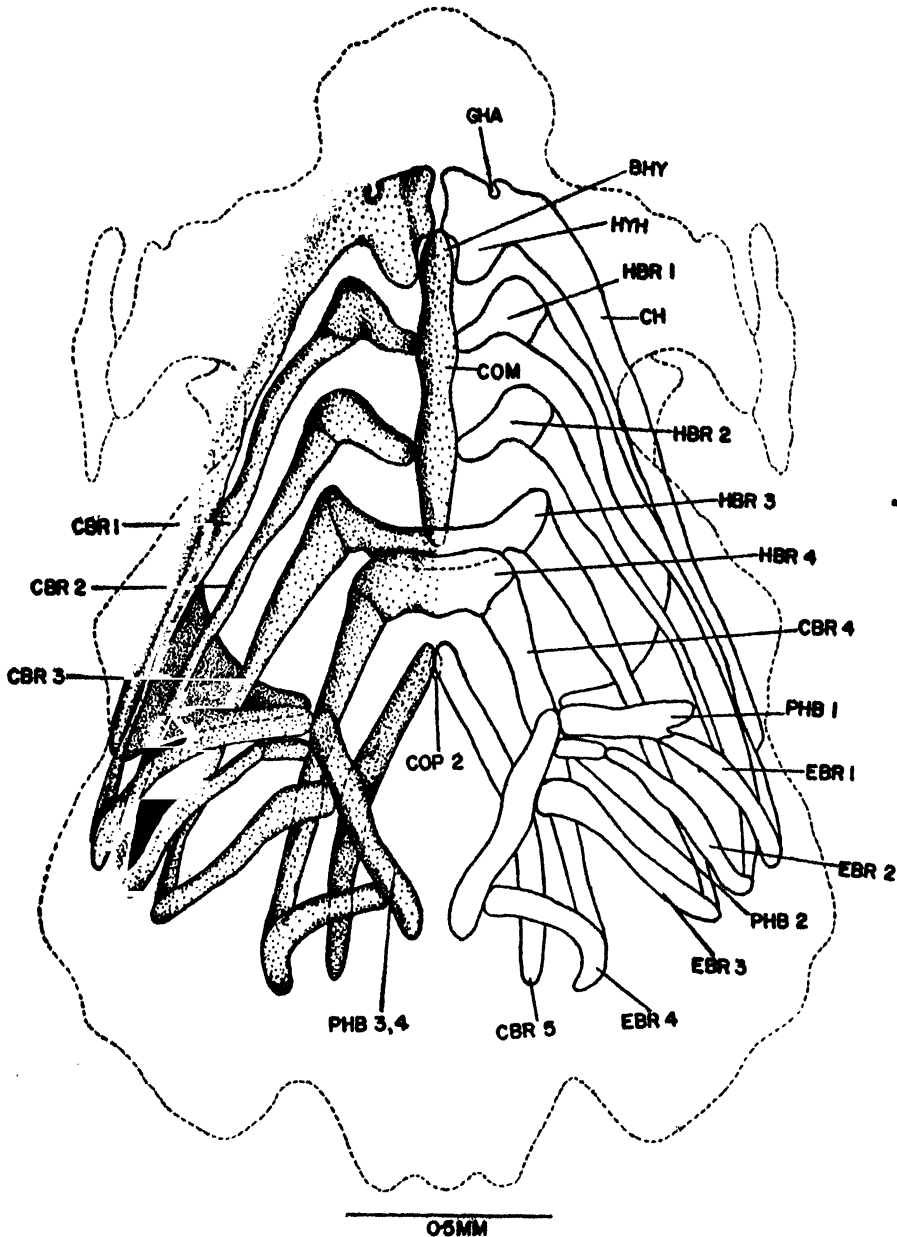


FIG. 6. Dorsal view of the hyobranchial skeleton of the fully formed chondrocranium of *Pangasius pangasius*, 17 mm. stage, and the outline of the chondrocranium (in broken line).

All the five branchial arches are well developed as in *Silonia*. The copula communis (Fig. 6, COM) is a thick cylindrical rod swollen anteriorly representing the basihyal and fused basibranchials. The first three branchial arches are in connection with the copula, but the fourth one arises posterior to the copula. In connection with the fifth branchial arch there appears to be a small rod of cartilage

representing the basibranchial cartilage (COP 2). The first four branchial arches are complete in having distinct hypo-, cerato-, epi- and pharyngobranchs. The pharyngobranchs of the first (PHB 1) and second (PHB 2) branchial arches are separate, whereas the pharyngobranchs of third and fourth (PHB 3, 4) are fused. The hypobranchs of third (HBR 3) and fourth (HBR 4) arches are more dorsally placed to the copula and are fused in the middle line.

The chondrocranium of *Ailia coila*

(16 mm. stage; head-length 3.0 mm.; Figs. 7 and 8)

The chondrocranium of *Ailia* is elongated and is very much different from the other two members *Silonia* and *Pangasius* studied.

The chondrocranium is fully formed in a 16 mm. fry of *A. coila*. The ethmoid region is curiously modified and is considerably elongated and rounded in the anterior end. The lamina precerebralis (Figs. 7, 8, LPC) is very much reduced and continues backwards as an internasal septum very much thinned out in the middle region. Arising from the antero-lateral corners of the lamina precerebralis is a pair of long narrow plate-like cartilages (NAC) extending backwards to the posterior end of nasal septum (Fig. 8, NS) called by Kindred (1919) the nasal alar cartilages. These cartilages form a sort of roof for the nasal organs in this region and the nasal barbels arise from the middle region of these alar cartilages. In describing the nasal alar cartilages of *Amiurus*, Kindred (1919) stated 'this has no connexion with the chondrocranial cartilage and has been named the "Nasenflügelknorpel" in the Characinidae by Sagemehl (1885) who regarded it as the phylogenetic remnant of the nasal-flap of the Selachians (p. 31)'.

The olfactory foramen (Fig. 8, AOF) is large and bears the same relation to the surrounding cartilages as noticed in *Silonia* and *Pangasius*. The lamina orbitonasalis (Figs. 7, 8, LON) connecting the orbital cartilages (ORC) and the ethmoid plate (Fig. 7, EP) has an anterior extension (Figs. 7, 8, ALON) which raises slightly upwards and forms a side wall for the nasal capsule. The solum nasi (Fig. 7, SN) is a thin plate of cartilage and forms the floor of the nasal capsules. The lamina orbitonasalis which is not very thick and large as seen in *Silonia* and *Pangasius* forms the posterior boundary for the nasal organs. The pterygoid process (Figs. 7, 8, PTP) is very broad and intimately connected with the lamina orbitonasalis. The sphenoseptal commissure (Fig. 7, SSC) is short and connects dorsally the posterior region of the nasal septum (NS) with the lamina orbitonasalis. The foramen orbitonasale is obliquely disposed and gives exit to the superficialis branch of the trigeminal nerve.

In the orbito-temporal region, the orbital cartilages are much reduced and the preoptic root (Fig. 8, POR) together with the trabecula communis is not covered dorsally in front of the hypophysial fenestra (Fig. 7, FH). A preoptic fontanelle seen in *Pangasius* is absent in *Ailia*, and there is a separate foramen for the passage of the oculomotor nerve (Fig. 8, FO) to innervate the eye muscles in the orbital cartilages. The orbital cartilages are not widely separated and are connected by a broad epiphysial bar (Figs. 7, 8, EPB) having a tiny taenia tecti medialis (Fig. 7, TTM) projecting into the posterior fontanelle. The hypophysial fenestra is more or less small and rounded in shape. On either side of the hypophysial fenestra between the plate-like trabeculae and the orbital cartilages of each side is a sphenoid fontanelle (Figs. 7, 8, SF) through which optic, trigeminal, abducens and facial nerves pass. The plate-like trabecula posteriorly becomes rod-like and is continuous with the basal plate.

The carotid artery enters the cranial cavity by a separate foramen (Fig. 7, FC) in the middle region of the trabecular plate. This condition is a slight deviation from the typical way the carotid artery passes medial to the trabecula in *Silonia* and *Pangasius*.

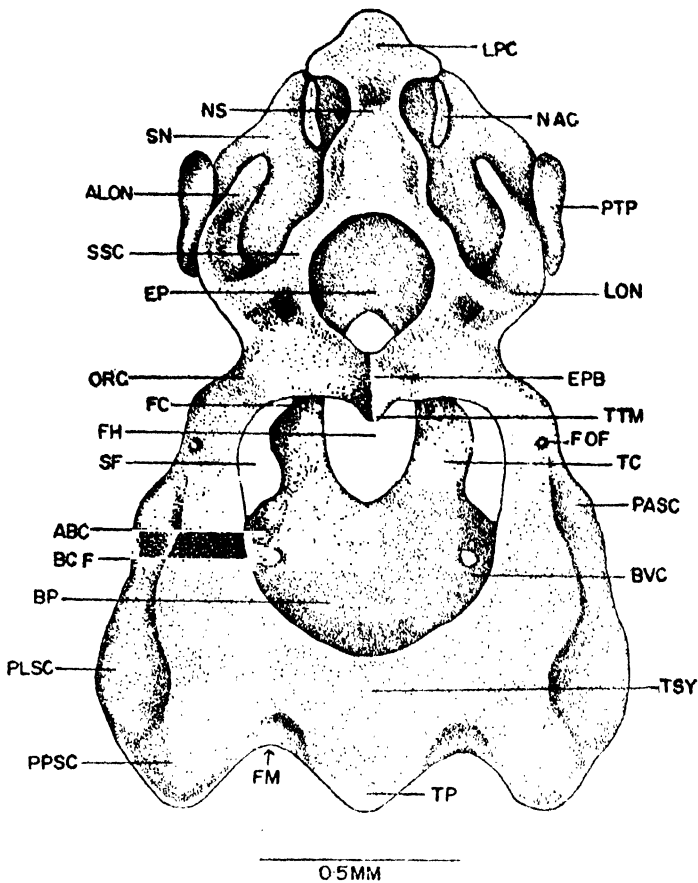


FIG. 7. Dorsal view of the fully formed chondrocranium of *Ailia coila*, 16 mm, stage (wax model).

The auditory region does not show any special features. The auditory capsules are not widely separated as in the other two genera studied. The external demarcation of the semicircular canals are not very distinct. The cavum labyrinthii communicates with the cavum cranii extensively except in the anterior and posterior parts where the auditory capsules possess medial walls. The oticus branch of the facial nerve passes through a foramen (Figs. 7, 8, FOF) in the postorbital process of the auditory capsule. The auditory capsules are united dorsally by a thin tectum synoticum (Fig. 7, TSY) which is fused with the tectum posterius (TP) of the occipital arch as in *Silonia* and *Pangasius*.

The basal plate (Fig. 7, BP) is in the form of a trough fused with the auditory capsule extensively except for the small basicapsular fenestra (BCF) which could be made out in the middle region, extending to a few sections, and the anterior basicapsular commissure (ABC) is noticed in front of the basicapsular fenestra. The glossopharyngeal and vagus nerves pass through a common fissura metotica (FM) as in the other cases studied. The notochord does not extend into the hypophysial fenestra or into a separate basicranial fenestra. The notochord is dorsal anteriorly and becomes medial to the basal plate posteriorly and is slightly triangular in transverse section.

Visceral arches.—In the visceral arch skeleton, the pterygoid process (Figs. 7, 8, PTP), on either side of the ethmoid region, chondrifies independently and is in

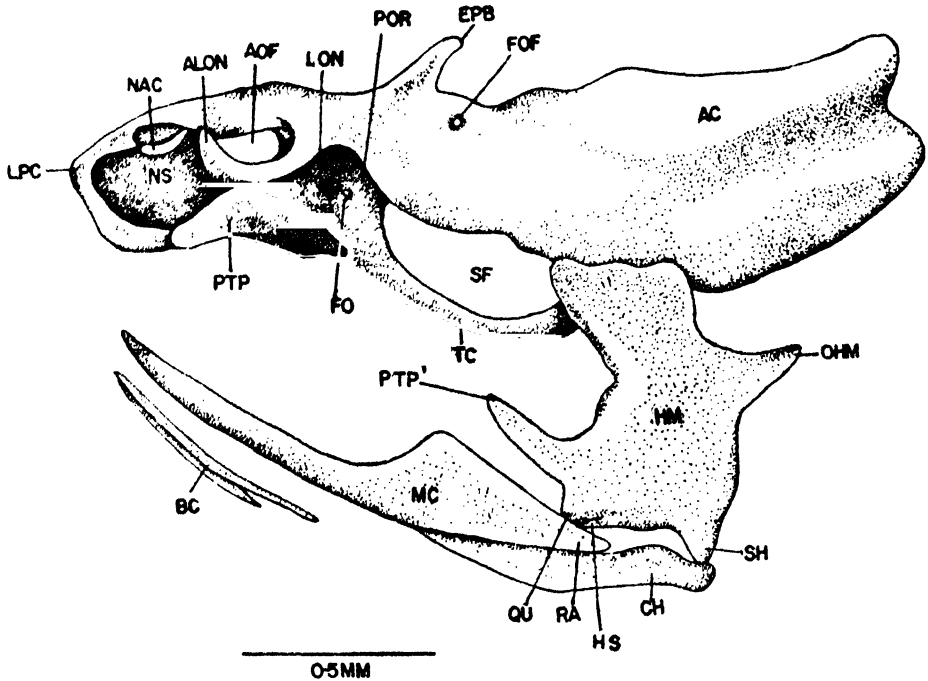


FIG. 8. Lateral view of the fully formed chondrocranium of *Ailia coila*, 16 mm. stage.

the form of a vertical plate narrow at both ends. The plate-like pterygoid process is intimately attached to the lamina orbitonasalis on each side and there are no articulating processes. The posterior portion of the pterygoid process which is discontinuous with the anterior portion is fused with the hyomandibula (Fig. 8, HM) and is pointed anteriorly. The quadrate (QU) is also fused with the hyomandibula and gives articulation to Meckel's cartilage (MC). The retroarticular process of Meckel's cartilage (RA) is more pronounced than that in *Silonia* and *Pangasius*.

The hyomandibula is a vertical plate-like cartilage articulating with the auditory capsule in the region of the lateral semicircular canal. The auditory capsule does not project in the form of a shelf over the region of articulation of the hyomandibula. Extending from the ventral portion of the hyomandibula adjacent to the quadrate is a tiny piece of cartilage, which may be recognized as the symplectic (Fig. 8, HS) extension of the hyomandibula, and which is not observed in the other siluroids studied. There is a niche for the passage of ramus hyomandibularis facialis in front of the middle region of the hyomandibula. Ventrally the hyomandibula is connected with the ceratohyal (CH) by a short interhyal (SH) which is fused with the hyomandibula.

All the five branchial arches are developed and the copula is a continuous rod, broad posteriorly. The first four branchial arches are developed in connection with the copula and the fifth arch arises at the posterior end of the copula. The first pharyngobranch is represented by a broad plate-like cartilage extending from the rod-like epibranch. The second pharyngobranch is small and closely attached to the first. The third pharyngobranch is in the form of a rod-like cartilage and is fused with the large pharyngobranch of the fourth arch. The fifth arch is represented by ceratobranchs and is free from the copula.

DISCUSSION

The chondrocrania of *Silonia*, *Pangasius* and *Ailia* (Schilbeidae, Siluroidea) conform to the other siluroids described, viz. *Amiurus* (Kindred, 1919) and *Ictalurus*

(Ryder, 1887), in their general plan, but differ in a number of points from the other siluroids and also amongst themselves.

The ethmoid plate formed by the fusion of the trabeculae is flat in the 8 mm. stage of *Silonia* and becomes arched in later stages. But it is trough-like in the 10 mm. stage of *Amiurus* (Kindred, 1919) and also in other teleosts like Clupeidae (Norman, 1926). The olfactory foramen is large and is bordered dorsally by the sphenoseptal commissure connecting the lamina precerebralis and the orbital cartilage, ventrally by the ethmoid plate, anteriorly by the lateral edge of the lamina precerebralis and posteriorly by the lamina orbitonasalis in *Silonia*, *Pangasius* and *Ailia* resembling *Amiurus* (Kindred, 1919). The ethmoid plate projects anteriorly in the form of massive ethmoid cornua in *Amiurus* (Kindred, 1919), but in *Ailia* they are absent, and in *Pangasius* and *Silonia* they are seen as slight projections. In *Gasterosteus* (Swinerton, 1902) the ethmoid cornua (pre-ethmoid cornua) give attachment to the anterior ends of the pterygoid process not noticed in *Amiurus* (Kindred, 1919).

Usually in most teleosts the forebrain during the development is situated on or above the ethmoid plate and gradually, by the development of an internasal septum (mesethmoid cartilage of Norman, 1926; septum nasi of Gaupp, 1906; septum rostri of Veit, 1911) separating the nasal fossae, it recedes. But this cartilaginous nasal septum (lamina precerebralis) is stretched out in the transverse plane in the 10 mm. stage of *Amiurus* (Kindred, 1919) and gradually in the 32 mm. stage the lamina precerebralis gives rise to an internasal septum. The nasal septum is massive in *Cyclopterus* (Uhlmann, 1921), *Galaxias* and *Callionymus* (Norman, 1926).

It is interesting to note that in *Amia* (Pehrson, 1922) the nasal septum in the 12 mm. stage arises as a short median process from the ethmoid plate between the olfactory nerves, and this process is broad posteriorly and in the 19.5 mm. stage the process becomes higher anteriorly. But in the 21.5 mm. stage this process has entirely disappeared with a large part of the hinder portion of the septum. In *Anguilla* (Norman, 1926) the nasal septum (mesethmoid cartilage) is suppressed, and subsequently a bone in its place is formed. Norman is of the opinion that the suppression of the mesethmoid cartilage in *Anguilla* might be due to the inward growth of the nasal organs which enlarge as the metamorphosis advances. Synbranchidae appear to present a sort of specialized condition with regard to the relationship between the ethmoid plate (trabecula communis) and the nasal septum. In *Siphonostoma* (Norman, 1926) the nasal septum appears late in the development. In *Hippocampus* (Kadam, 1957) an internasal septum is noticed in contact with the ethmoid plate in the 9 mm. stage and the nasal septum continues to remain distinct in the older stage (18 mm. stage), thus indicating its independent origin. In *Syngnathus* (Kindred, 1921, 1924) further specialization of this cartilage is observed, and the nasal septum is completely eliminated from the early stages in the development of chondrocranium. However, in the 45 mm. stage the nasal septum arises in the posterior region by the fusion of the ectethmoid cartilages.

In describing the ethmoid region of *Silonia* and *Pangasius* I have preferred to retain the term lamina precerebralis to the nasal septum as at no stage the lamina precerebralis extends backwards in the form of a nasal septum as seen in the 32 mm. stage of *Amiurus* (Kindred, 1919). The lamina precerebralis is massive in *Silonia* and *Pangasius* stretched out in a transverse plane and forms the anterior boundary for the cranial cavity. The nasal fossae communicate with each other to a great extent through the olfactory foramina. It may be suggested that this condition is also a specialized feature in the chondrocranium. However, in *Ailia* the lamina precerebralis becomes thinned out between the nasal capsules, and extends backwards as the internasal septum.

In *Silurus*, *Callichthys*, *Choetostomus* and *Labrus* (Pollard, 1895), *Syngnathus* (Kindred, 1921), *Sebastes* (Mackintosh, 1923), and *Ophicephalus* (Srinivasachar,

1953) a median rostral cartilage is developed in front of the ethmoid plate as a premandibular element. But Berril (1925) has shown that the rostral cartilage in *Pleuronectes* and *Solea* arises as paired rudiments. Such a rostral cartilage is absent in *Pangasius*, *Silonia* and *Ailia*. However, labial cartilages supporting the two mandibular barbels on either side are noticed in *Ailia*, as in *Ictalurus* (Ryder, 1887), and in *Pangasius* and *Silonia* they are absent.

Kindred (1919), in describing the 32 mm. stage chondrocranium of *Amiurus*, has shown that the roof of the nasal fossa is formed by an independent 'nasal alar' cartilage supporting the nasal barbel and having no connection with the chondrocranium. Sagemehl (1885) has also noticed similar cartilage in Characinidae and regarded it as the phylogenetic remnant of the nasal-flap cartilage of the Selachians. Similar cartilages have been observed by me in *Ailia* extending posteriorly from antero-dorsal portion of the nasal septum and appear to have connection with the nasal septum by means of connective tissue. Further, it has been noticed that the nasal barbel arises from the middle region of the nasal alar cartilages and is fused at the region of attachment to it. The nasal alar cartilages are absent in *Pangasius* and *Silonia*.

The lamina orbitonasalis (ectethmoid cartilage) arises generally in teleosts as postero-lateral outgrowths from the ethmoid plate forming more or less the posterior boundary for the nasal capsules separating them from the orbits. The dorsal edge of the lamina orbitonasalis of each side is fused with the posterior part of the nasal septum or the antero-lateral edge of the orbital cartilages. These laminae orbitonasales are broad and massive in *Salmo* (Gaupp, 1906), *Cyclopterus* (Uhlmann, 1921), but slender and rod-like in *Anguilla* (Norman, 1926) and *Ophicephalus* (Srinivasachar, 1953). In teleosts like *Salmo* (de Beer, 1937) and *Amiurus* (Kindred, 1919) the lamina orbitonasalis is pierced by a foramen for the passage of the ophthalmic branch of the trigeminal nerve. But Norman (1926) failed to notice a foramen in the lamina orbitonasalis of a 25 mm. stage of Salmon and the ophthalmic nerve passes dorsal and external to the lamina as in *Anguilla* (Norman, 1926) and *Clupea* (Wells, 1922). In *Ophicephalus* (Srinivasachar, 1953) the ophthalmic branch passes through a notch on the dorsal surface of the lamina orbitonasalis.

In Syngnathidae (*Syngnathus*, Kindred, 1921; *Siphonostoma*, Norman, 1926; *Hippocampus*, Kadam, 1957) the lamina orbitonasalis arises as an independent cartilage not connected with the ethmoid plate.

In *Silonia*, *Pangasius* and *Ailia*, the lamina orbitonasalis is thick and massive and forms a posterior boundary for the nasal capsule. The lamina orbitonasalis in these forms possesses an anterior extension which serves as a lateral wall for the posterior part of the nasal sacs. The lamina orbitonasalis is thin in the 8 mm. stage of *Silonia* and gradually it becomes thick and massive in the 18 mm. stage. In all the three forms, *Silonia*, *Pangasius* and *Ailia*, an obliquely disposed foramen is seen in the lamina orbitonasalis for the passage of the ophthalmic branch of trigeminal nerve as in *Amiurus* (Kindred, 1919). This appears to be a general feature of the siluroids. The anterior extension of the lamina orbitonasalis is considerably lengthened and is also bent upwards in *Ailia*. A preoptic fontanelle is noticed behind the lamina orbitonasalis in *Pangasius* and *Silonia* as in *Amiurus* (Kindred, 1919). The ventral face of the lamina orbitonasalis forms the articular surface for the pterygoid process in *Pangasius* and *Silonia* as in *Amiurus* (Kindred, 1919).

A distinct sphenoseptal commissure connecting dorsally the nasal septum (lamina precerebralis) and the lamina orbitonasalis is developed in *Ailia*, *Pangasius* and *Silonia* as in *Salmo* (de Beer, 1937).

The eye muscle canals (myodomes) seen in teleosts like *Salmo* (de Beer, 1937), *Clupea* (Wells, 1922), *Cyclopterus* (Uhlmann, 1921) and *Gambusia* (Ramaswami, 1945) is absent in *Silonia*, *Pangasius* and *Ailia* as also in *Amiurus* (Kindred, 1919). The absence of the myodome appears to be a uniform character in the skull of most of the siluroids. It is obvious that the mode of attachment of the recti muscles and

the presence or absence of the myodome depend on the size of the eyes of the adult fish. In fishes where the eyes are large the myodomes are well developed and in those which possess relatively small eyes the myodome is absent. However, Bhimachar (1933) has observed a small myodome in one of the siluroids, *Silandia*. The rectus muscles in *Silonia*, *Pangasius* and *Ailia* are attached to the lateral surface of the trabeculae in the posterior region of the orbit as in *Amiurus* (Kindred, 1919).

The orbital cartilage which forms the lateral boundary in the orbito-temporal region in most of the siluroids studied is in the form of a vertical plate-like cartilage in front of the extensive sphenoidal fissure through which the optic, oculomotor, trigeminal and facial nerves pass in *Pangasius*, *Silonia* and also in *Amiurus* (Kindred, 1919). Posteriorly the preoptic root of the orbital cartilage continues as the postorbital process to fuse with the anterior end of the auditory capsule. In *Ailia*, a separate foramen for the exit of the oculomotor nerve is noticed in the preoptic root of the orbital cartilage. A preoptic fontanelle is noticed in *Amiurus* (Kindred, 1919). I have also described it in *Pangasius*; however, it is absent in *Ailia* and *Silonia*.

The orbital cartilages become discontinuous from the lamina orbitonasalis in some stage or the other in the development of chondrocranium in a number of teleosts like *Gasterosteus* (Swinerton, 1902), *Sebastes* (Mackintosh, 1923) and *Ophicephalus* (Srinivasachar, 1953). But in *Silonia*, *Pangasius* and *Ailia* the orbital cartilages at no stage become discontinuous.

The trabeculae in the orbito-temporal region are also short plate-like cartilages surrounding the extensive hypophysial fenestra, forming the ventral border of the sphenoid fissure described above in *Silonia*, *Pangasius* and *Ailia* as in *Amiurus* (Kindred, 1919). The trabeculae in front of the hypophysial fenestra are fused to form the trabecula communis (ethmoid plate), and an interorbital septum is absent in *Silonia*, *Pangasius* and *Ailia* and also in *Amiurus* (Kindred, 1919); hence the chondrocranium is of the platytrabec type. This type is characteristic of most Selachians and occurs also in *Amia* (Pehrson, 1922), *Ophicephalus* (Srinivasachar, 1953), and according to Norman (1926) among other teleosts the platytrabec chondrocranium is found only in Siluroidea and in *Homaloptera* among the Cyprinoidea (Sagemehl, 1891) and he considers that the condition might be a secondary one in these fishes. In other teleosts like *Salmo* (de Beer, 1937), *Sebastes* (Mackintosh, 1923), and *Anguilla* (Norman, 1926), a tropitrabec type of chondrocranium is noticed. The trabeculae which are continuous with the parachordals in the 10 mm. stage of *Amiurus* (Kindred, 1919) become interrupted in the 32 mm. stage. But in *Silonia*, *Pangasius* and *Ailia* the discontinuity of the trabeculae has not been observed in any stage of the development studied.

The carotid artery enters the cranial cavity in a typical way passing through the basicranial fenestra medially to the trabecula through a notch in the 10 mm. stage of *Amiurus* (Kindred, 1919). A similar condition is also noticed in *Silonia* and *Pangasius*, but the notch is absent. However, in *Ailia*, a distinct foramen is observed in the middle region of trabecular plate as in *Gymnarchus* (Assheton, 1907). In *Anguilla* (Norman, 1926) a single foramen for the internal carotid artery is noticed as in *Squalus* (van Wijhe, 1922).

Generally in most of the teleosts the lateral wall in the chondrocranium is formed by the orbital cartilages (taenia marginalis) which lie between the brain and the upper parts of the eyes, and extend between the auditory capsules and the nasal septum. In most forms like *Clupea* (Wells, 1922), *Anguilla* (Norman, 1926), *Salmo* (de Beer, 1937) and others, the orbital cartilages are continuous bars connecting the auditory capsules with the ethmoid region, but in some of the members as in *Gasterosteus* (Swinerton, 1902), *Sebastes* (Mackintosh, 1923) and *Ophicephalus* (Srinivasachar, 1953) the orbital cartilages become discontinuous, the ethmoid and auditory regions being separate. An extreme case is seen in *Syngnathidae*

(Norman, 1926) where they are practically non-existent, and the anterior and posterior ends are observed as small processes extending from the auditory capsules and the lamina orbitonasalis respectively. In most of the siluroids studied, the orbital cartilages are very well developed and are in the form of large vertical plates and in *Amiurus* Kindred (1919) refers to these as alisphenoid cartilages.

The two orbital cartilages are interconnected in the middle region by an epiphysial bar forming a sort of roof in the orbital region, noticed in most teleosts like *Salmo* (de Beer, 1937), *Exocoetus* (Lasdin, 1913), *Clupea* (Wells, 1922), *Gambusia* (Ramaswami, 1945), *Ophicephalus* (Srinivasachar, 1953) and others. A similar epiphysial bar is observed in *Silonia*, *Pangasius* and *Ailia*, but the bar is much thicker in *Ailia* when compared with the other two genera.

A lateral commissure formed by the fusion of postpalatine and preotic processes connecting the lateral edge of the basal plate with the anterior wall of the auditory capsule forms the ventro-lateral wall of the trigeminofacialis chamber in *Syngnathus* (Kindred, 1921), *Anguilla* (Norman, 1926), *Salmo* (de Beer, 1937) and *Gambusia* (Ramaswami, 1945). Such a lateral commissure is absent in *Silonia*, *Pangasius* and *Ailia* as in *Amiurus* (Kindred, 1919). The absence of a lateral commissure appears to be a general feature in the chondrocranium of siluroids, because the trigeminal and facial nerves together with the optic and abducens nerves pass through a sphenoid fissure bordered dorsally by the orbital cartilage, ventrally by the trabecula, anteriorly by the preoptic root of the orbital cartilage and behind by the basal plate.

The auditory capsules of *Silonia*, *Pangasius* and *Ailia* are very much similar to a typical teleostean condition as seen in *Salmo* (de Beer, 1937), *Amiurus* (Kindred, 1919) and others. The oticus branch of the facial nerve passes through the anterior region of the auditory capsule to innervate the lateral line sensory canal in *Silonia* and *Pangasius*, but the same nerve passes through the postorbital process of the orbital cartilage in *Ailia* as in *Amiurus* (Kindred, 1919). The cavity of the otic capsule communicates with the cavum cranii by a wide fenestra in *Amiurus* (Kindred, 1919) as in larval *Acanthias* (Sewertzoff, 1889). Though in *Silonia*, *Pangasius* and *Ailia* there is a large opening in the middle of the auditory capsule into the cranial cavity, a medial wall, however, can be observed in the anterior and posterior ends of the capsule.

The auditory capsules are united with the parachordals by means of anterior and posterior basicapsular commissures leaving large fenestrae as in *Salmo* (de Beer, 1937). The anterior basicapsular commissure can always be recognized behind the facial nerve and the posterior basicapsular commissure behind the glossopharyngeal nerve and in front of the vagus nerve as in *Salmo* (de Beer, 1937) and other teleosts. In *Silonia* the basicapsular fenestra is absent as in *Amiurus* (Kindred, 1919), but in *Pangasius* the anterior basicapsular fenestra is more pronounced, while in *Ailia* it extends only to a few sections. In *Silonia*, *Pangasius* and *Ailia* both the glossopharyngeal and vagus nerves pass through a common fissure—the metotic fissure—and the commissure in front of the metotic fissure represents the basivestibular commissure as in *Clupea* (Wells, 1922), *Syngnathus* (Kindred, 1921), *Ictalurus* (Ryder, 1887) and *Ophicephalus* (Srinivasachar, 1953). But in *Amiurus* (Kindred, 1919) the glossopharyngeal and vagus nerves pass through independent foramina as in *Salmo* (de Beer, 1937) and *Anguilla* (Norman, 1926).

Dorsally the two auditory capsules are united by a tectum synoticum which is fused with tectum posterius of the occipital arch in *Silonia*, *Pangasius* and *Ailia* as in *Amiurus* (Kindred, 1919). However, a tectum posterius is absent in other teleosts like *Salmo* (de Beer, 1937), *Gambusia* (Ramaswami, 1945) and *Ophicephalus* (Srinivasachar, 1953).

In the visceral arch skeleton the pterygoquadrate is curiously modified in *Silonia*, *Pangasius* and *Ailia* as in *Amiurus* (Kindred, 1919). The pterygoid process chondrifies independently and remains so throughout the development of

the chondrocranium in all the three members studied. A small ethmopalatine process of the pterygoid process articulating in the region of lamina orbitonasalis is noticed in the 8 mm. stage of *Silonia* and this process gradually becomes reduced as development advances and in the fully formed chondrocranium the pterygoid process practically loses the ethmopalatine portion. But in other forms such a process is not noticed in the chondrocranium. However, a rostralpalatine process has not been observed in any of the forms studied and the pterygoid process does not extend beyond the middle region of the ethmoid plate.

But in *Amia* the pterygoid process chondrifies separately from the main cartilage and is connected with it by procartilage and subsequently becomes continuous with the quadrate (Pehrson, 1922). In *Clupea* (Wells, 1922) and *Ophicephalus* (Srinivasachar, 1953), where the pterygoid process chondrifies independently, it gradually extends backwards and becomes fused with the body of the quadrate cartilage. However, Norman (1926) disagrees with Wells (1922) with regard to the development of mandibular arch in *Clupea* and is of the opinion that the quadrate is distinct from the hyomandibula.

Further, the body of the quadrate is fused with the hyomandibula in *Silonia*, *Pangasius* and *Ailia* as in other siluroids, *Amiurus* (Kindred, 1919), *Ictalurus* (Ryder, 1887) and other fishes like *Clupea* (Wells, 1922) and *Ophicephalus* (Srinivasachar, 1953). It appears to be a general feature in the chondrocranium particularly in siluroids that the quadrate portion of the first visceral arch is fused with the hyomandibula, a portion of the second visceral arch.

Generally in teleosts the hyomandibula articulates with the auditory capsule in the region of the lateral semicircular canal and is in the form of a plate-like cartilage and is distinct from the quadrate as in *Gasterosteus* (Swinerton, 1902), *Syngnathus* (Kindred, 1921), *Salmo* (de Beer, 1937) and others. In all the above cases the hyomandibular cartilage possesses a foramen for the passage of the hyomandibular branch of the facial nerve and a distinct symplectic extension is noticed projecting ventrally from it. But in *Silonia*, *Pangasius* and *Ailia* the plate-like hyomandibula is fused with the quadrate ventrally and does not possess a symplectic extension, though an apparent tiny projection extending from its ventral side and adjacent to the quadrate is seen in *Ailia*. The hyomandibular branch of the facial nerve passes through a deep gap in front of the hyomandibula in *Silonia*, *Pangasius* and *Ailia* and not through a distinct foramen as in *Amiurus* (Kindred, 1919). However, in the older stages examined (23 mm. stage) of *Silonia* the nerve passes out through a foramen in the bone.

The branchial arches in *Silonia*, *Pangasius* and *Ailia* develop in the same way as in other teleosts studied, and in *Pangasius* in connection with the fifth ceratobranch a tiny rod corresponding to the basibranchial is noticed.

SUMMARY AND CONCLUSION

1. Three stages in the development of the chondrocranium in *Silonia silondia* and one stage in each of *Pangasius pangasius* and *Ailia coila* are described.

2. In the 8 mm. stage of *Silonia* the chondrocranium is elongated and possesses a large hypophysial fenestra into which projects the notochord. Independent chondrification of pterygoid process and the fusion of hyomandibula and quadrate are noticed. In 11 mm. stage, an epiphysial bar, tectum synoticum and tectum posterius are developed.

3. The chondrocranium is fully formed in 18 mm. stage of *Silonia* and is compared with similar stages of *Pangasius* and *Ailia*. The important similarities in the chondrocranium of *Silonia*, *Pangasius* and *Ailia* are as follows:

The sphenoseptal commissures connect the orbital cartilages with the laminae orbitonasales. The solum nasi forms complete floor for the nasal sacs. The anterior extension of the lamina orbitonasalis is quite extensive and forms the

lateral wall for the nasal sac. The sphenoid fissure gives exit to optic, trigeminal, abducens and facial nerves. A lateral commissure forming the anterior boundary for the trigeminofacial chamber is absent. The tectum synoticum connecting the auditory capsules is continuous with the tectum posterius of occipital arches. There is no membranous or cartilaginous interorbital septum and the chondrocranium conforms to the platyttrabic type.

In the visceral arch skeleton, the pterygoid process chondrifies independently and is unconnected with the quadrate. The quadrate is fused with hyomandibula. The hyomandibular branch of the facial nerve passes in a gap in front of the hyomandibula.

4. The chondrocranium of *Silonia* is very much similar in most respects to, and differs in some minor characters from, the chondrocranium of *Pangasius*. But the chondrocranium of *Ailia* differs considerably from the other two members, *Silonia* and *Pangasius*, and the peculiar features in the chondrocranium can be summarized as follows:

A distinct nasal septum is noticed and nasal alar cartilages are developed extending from the antero-lateral corners of the nasal septum. The oculomotor nerve gains a separate exit in the preoptic root of orbital cartilage and otic branch of facial nerve passes in the postorbital process of the orbital cartilage. In the visceral arch skeleton a symplectic extension of the hyosymplectic cartilage is seen extending to a few sections. Meckel's cartilages unite and fuse anteriorly.

5. In conclusion it may be suggested here that *Silonia* and *Pangasius* on account of their close similarity in their chondrocranial characters may be included in the same genus *Pimelodus*, but as two different species, as was done by Hamilton (1822) in his book on the 'Gaugetic fishes'.

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KEY TO THE LETTERING

ABC	..	anterior basicapsular commissure;
AC	..	auditory capsule;
ALON	..	anterior extension of the lamina orbitonasalis;
AOF	..	olfactory foramen;
BC	..	barbel cartilage;
BCF	..	basicapsular fenestra;
BHY	..	basihyal;
BP	..	basal plate;
BVC	..	basivestibular commissure;
CBR 1	}	.. ceratobranchials 1-5;
CBR 2		
CBR 3		
CBR 4		
CBR 5		
CH	..	ceratohyal;
COM	..	copula communis;
COP 2	..	copula in connection with the fifth ceratobranch;
EBR 1	}	.. epibranchials 1-4;
EBR 2		
EBR 3		
EBR 4		
EP	..	ethmoid plate;
EPB	..	epiphysial bar;
FO	..	foramen for the carotid artery;
FH	..	hypophysial fenestra;
FM	..	fissura metotica;
FON	..	foramen orbitonasale;

FOF	..	foramen for the oticus branch of the facial nerve;
FO	..	foramen for the oculomotor nerve;
GHA	..	groove in the hypohyal for the passage of the hyoidean artery;
HBR 1	}	hypobranchials 1-4;
HBR 2		
HBR 3		
HBR 4		
HM	..	hyomandibula;
HS	..	hyosymplectic;
HYF	..	niche in the hyomandibular cartilage for the passage of the ramus hyomandibularis facialis;
HYH	..	hypohyal;
LON	..	lamina orbitonasalis;
LPC	..	lamina precerebralis;
MC	..	Meckel's cartilage;
NAC	..	nasal alar cartilage;
NC	..	notochord;
NS	..	nasal septum;
OA	..	occipital arch;
OHM	..	opercular process of the hyomandibular cartilage;
ORC	..	orbital cartilage;
PASC	..	prominence of the anterior semicircular canal;
PC	..	parachordal;
PHB 1	}	pharyngobranchials 1 and 2;
PHB 2		
PHB 3, 4	..	fused pharyngobranchials 3 and 4;
PLSC	..	prominence for the lateral semicircular canal;
PPSC	..	prominence for the posterior semicircular canal;
POF	..	preoptic fontanelle in the preoptic root of the orbital cartilage;
POP	..	postorbital process of the auditory capsule;
POR	..	preoptic root of the orbital cartilage;
PTP	..	pterygoid process;
PTP'	..	processus pterygoideus of the quadrate;
QU	..	quadrate;
RA	..	retroarticular process of Meckel's cartilage;
SE	..	sphenoid fissure;
SH	..	interhyal;
SN	..	solum nasi;
SSC	..	sphenoseptal commissure;
TC	..	trabecula;
TP	..	tectum posterius;
TSY	..	tectum synoticum;
TTM	..	taenia tectomedialis.

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THE OCCURRENCE AND ACTIVITIES OF CERTAIN BACTERIAL GROUPS IN OFF-SHORE MARINE ENVIRONMENTS OF GULF OF MANNAR, OFF TUTICORIN¹

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INTRODUCTION

Very little work has been done on the marine bacterial groups in off-shore regions in India, though comprehensive accounts are available about these in the U.S.A. (ZoBell, 1946). Earlier, Waksman and collaborators (1933a) demonstrated the presence of certain physiological groups, such as cellulose digesters, algin digesters, agar digesters, denitrifiers and 'copper bacteria', in the Gulf of Maine. Some workers have studied single groups, such as chitin digesting bacteria (reviewed by Sreenivasan, 1955), agar digesting bacteria (Lundestad, 1928; Hidake and Samesima, 1953), sulphate reducing bacteria (ZoBell and Rittenburg, 1948), cellulose digesting bacteria (Stanier, 1940; Waksman *et al.*, 1933a), algin digesting bacteria (Waksman *et al.*, 1934), and urea splitting bacteria (ZoBell and Feltham, 1935). Wood (1953), in Australia, has also given an account of some of these groups in his paper which contains an excellent account of fish spoilage bacteria also. In our earlier work on the off-shore sea-water of the west coast, bare mention was made of the abundance of denitrifiers and absence of coliforms. Velankar (1955) has studied the presence of a few physiological groups, but only in the shallow inshore bay in the Gulf of Mannar. In connection with the study of the productivity of pearl banks and chank (conch-shell) grounds, investigation was carried out on the bacterial flora of sea-water, pearl oysters, chanks and bottom sand, 10 to 12 miles off Tuticorin. The periods of investigation were in September, 1953 and in January, 1955. The existence of these groups may explain the biological cycles in the sea, the deterioration of submerged materials, such as ship's bottoms, netting, etc., and spoilage of marine products.

EXPERIMENTAL PROCEDURES AND MATERIALS

The pearl banks were visited during the periods of pearl bank inspections in September, 1953, March, 1954 and January, 1955. Water samples were taken from south, north, central and eastern sections of Tolayiram par, and also from Kanava par and Kadayan par. The average of bacterial counts of these and those of the pearl oysters is indicated in Table I. The sand and chanks were collected from the chank beds near Tolayiram par. The temperature of water ranged from 27.3°C. to 29.5°C.

Water samples were collected 12" beneath the surface and from the bottom (8 to 12 fathoms depth), and plated out on board the vessel itself. A Nansen water-bottle was used and the water at once transferred to a sterile glass-stoppered bottle.

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TABLE I
Bacterial groups in different areas of pearl banks and the bottom materials, in the Gulf of Mannar

Bacterial groups	Sea-water (off-shore)						Chanks		Pearl oysters	
	September, 1953		March, 1954		January, 1955		Sand (off-shore)	1		11
	Surface	Bottom	Surface	Bottom	Surface	Bottom				
Total counts/c.c. or gm. on sea-water agar	392	33	<30	<30	456	23	99	TNC	>3,000,000	649,000
Total counts/c.c. or gm. on fresh-water agar	Spr.	26	Nil	Nil	Nil	Nil	Nil	>10,000	45,000	17
Coliforms, 'M.P.N.' per 100 ml.	Nil	Nil	Nil	Nil	Nil	Nil	Nil	1,000	1,000	1,000
Denitrifiers	+	+	+	+	+	+	+	+	+	+
Ureafermenters	+	+	+	+	+	+	+	+	+	+
Agar digesters	+	+	+	+	+	+	+	+	+	+
Algin digesters	+	+	+	+	+	+	+	1,000	1,000	1,000
Chitin digesters	+	+	+	+	+	+	+	1,000	1,000	1,000
Cellulose digesters	+	+	+	+	+	+	+	1,000	1,000	+
Sulphate reducers	+	+	+	+	+	+	+	7,300	+	5
'Copper bacteria'	+	+	+	+	+	+	+	+	+	+
+	= Present.	+	= Not tested.	+	= Rare.	TNC	= Too numerous to count.			
-	= Absent.	-	= Rare.	+	= Spreaders.	Spr.	= Spreaders.			

+ = Present.
 - = Absent.

.. = Not tested.
 ± = Rare.

TNC = Too numerous to count.
 Spr. = Spreaders.

Bottom sand was scooped out by a sampler devised by Chidambaram *et al.* (1951). Pearl oysters and chanks were taken out by naked divers and were handled on shore according to the usual procedures for the examination of shell fish (U.S. Public Health Service, 1946). Dilutions of sea-water, sand, chanks, etc., were inoculated into appropriate enrichment media for each of the physiological groups. When the presence of these groups was proved pure, cultures were isolated and representative strains were studied.

Bacterial population of sea-water.—The surface and bottom sea-water examined on a number of occasions and in different locations such as *Tolayiram par*, *Kanava par*, *Kadayan par*, etc., indicated that the bacterial populations were sparse, being a few score. There is no variation, in the numbers between different areas of off-shore sea. Marine sand, which mainly consisted of silica and calcareous shell fragments, did not harbour a rich population of bacteria, though very reactive types were isolated. The counts on ZoBell's medium were greater than on fresh-water agar. It was also noted that some of the bacteria failed to grow on salt-free media indicating a special ecological group, viz. 'marine' bacteria. A few could, by gradual laboratory cultivation, be 'trained' to grow on fresh-water media but a majority was halophilic. These mostly belonged to *Pseudomonas*, *Achromobacter*, *Bacterium*, and *Corynebacterium*. *Bacillus*, *Micrococcus* and *Sarcina* did not fail to grow on fresh-water media.

Bacterial action of sea-water.—The very low population of off-shore sea-water may be due to the inherent bactericidal property, especially towards the autochthonous organisms of soil, sewage, etc. In Tuticorin, the town sewage and the effluents from the Harvey Mills are let into the sea. A study was made of the influence of sea-water on the survival of the coliform organisms. This was necessary because the beds of shell fish, such as pearl oysters and chanks, are situated 8 to 12 miles off this discharge site. Further, edible fish are also caught nearby. Results tabulated in Table II clearly bring out the fact that even within a distance of half a mile the coliforms are drastically reduced in population and, at a distance of five miles, there is practically no coliform organism traceable. Vaccaro *et al.* (1950) also found that the number of bacteria decreases much more rapidly than could be accounted for by dilution alone. The 'Total' counts also were progressively reduced with the increasing distance from the land, indicating the inability of terrestrial flora to establish themselves in the open seas.

TABLE II

Coliform and total counts of the sewage outfall and of sea-water opposite

	Distance						
	Near the outfall opposite Harvey Mills	$\frac{1}{8}$ mile	$\frac{1}{2}$ mile	$1\frac{1}{2}$ miles	3 miles (near Haro Island)	5 miles	8 miles
September, 1953:							
Coliforms MPN/100							
c.c. . .	160,000	..	920	Nil
Total counts ..	60,000	..	390	18
March, 1954:							
Coliforms ..	1,600,000	1,600	..	49	49	4	Nil
Total counts ..	300,000	3,000	..	50	87	29	..
On (a) Sea-water,							
(b) Fresh-water agar	TNC	470	..	Spreader	27	18	..

PHYSIOLOGICAL GROUPS

Agar digesting bacteria.—2 to 3% of the colonies from sea-water and 7 to 10% from marine sand were agar digesters. They were profusely pigmented, pink, yellow orange to red, brown and also non-chromogenic. They all liquefied agar remarkably rapidly. On the agar plates, they initially showed a depression which widened into a saucer-shaped crater and finally spread out liquefying the entire plate. These agar digesters were slender, long, C- or S-shaped curved rods as well as pleomorphic, and straight sluggishly motile or non-motile, non-sporing and gram negative. Two cultures from sea-water were polar flagellated. The others probably belong to the genus *Bacterium*. 'Reducing sugars' were identified, when agar was liquefied. A description of the cultures is given in Table III. Great difficulty was experienced in preserving these cultures, since they die off rapidly in the liquefied (agar) medium, probably due to acid formation from the sugars. A large percentage of colonies isolated from chanks were also agar digesters. ZoBell (1946) computed that 1 to 2% of bacteria occurring in the sea are capable of agar digestion, while Wood (1953) found between 2 to 5% in Australian waters. Waksman *et al.* (1933a) noted them mostly in diatom tows. The cultures described by us in Table III differ from those described by Lundestad (1928).

Alginate acid digesters.—Bacteria digesting alginate acid were invariably present in 1 c.c. of sea-water, both surface and bottom, but to a smaller extent only in pearl oysters, and rarely in chanks and in sand. On algin agar plates, they cleared the opacity, formed transparent zones or 'halos' due to the utilization of alginates and even softened the agar in some cases. In liquid enrichment cultures gas was also produced. All the isolates studied were gram negative non-spore-forming peritrichous or non-motile rods, belonging to the genus *Bacterium*. Some of them could be identified with *Bacterium alginovorum* (Waksman *et al.*, 1934). A description of these is given in Table IV.

Cellulose digesting bacteria.—The sea-water and sand were devoid of bacteria digesting cellulose or utilizing it as a sole source of carbon. But the flesh of chanks (*Xancus pyrum*) and pearl oysters (*Pinctada vulgaris*) showed the presence of this group in 10^{-3} dilution. Growth of the organisms occurred on the filter paper partially dipping in the cellulose enrichment medium (Waksman *et al.*, 1933c) but only at the exposed and not at the submerged portion. Growth was evident in 2 days and in 4 days, the paper was cut up at the liquid-paper interface. Gradually the filter paper turned transparent due to digestion till it finally broke down. Purification of the culture was made by serial dilution. Two types of organisms were noted—one, a minute gram negative, non-sporing, actively motile, monotrichous rod, and the other, a very actively motile spindle-shaped organism, resembling *Cell-fasciula*.

Chitin digesters.—They were frequently recovered from chanks and pearl oysters, but less often from sand and sea-water. After enrichment and purification by serial dilution, the ability of these organisms to utilize chitin as the sole source of carbon and nitrogen was tested. Pure colonies were isolated on chitin agar plates and studied in detail. An account of one new species of 'marine' chitin digesting bacterium *Pseudomonas chitinovora* is given by Sreenivasan (1955). It was shown to solubilize chitin very rapidly, liberating ammonia. Subsequently many more cultures have been isolated and studied. All these were monotrichous gram negative rods belonging to *Pseudomonas*. Surprisingly, they were all denitrifiers, rapidly liberating nitrogen from nitrates. This explains the gas production observed in chitin enrichment media during primary isolation.

Copper bacteria.—Waksman *et al.* (1943) isolated some bacteria from sea-water and submerged slides tolerating a maximum of 200 mg./l. of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. In view of the rôle of film forming bacteria in fouling submerged materials (ZoBell, 1946) and their rôle in affecting the antifouling paints, the copper tolerance of

marine bacteria was studied by plating out sea-water, chank flesh and pearl oyster flesh on nutrient sea-water agar containing 250 p.p.m. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. The sea-water bacteria did not grow in this concentration of CuSO_4 , but a large number of colonies appeared from chanks (7,300 per gm.) and a few from pearl oysters (5 per gm.). After purification on CuSO_4 agar, pure cultures were studied in detail. These 'copper' bacteria formed brownish copper coloured colonies. Some tolerated even 1,000 p.p.m. of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, while four cultures from pearl oysters tolerated up to 750 p.p.m. This tolerance of high concentration of CuSO_4 is highly interesting, since very few workers have investigated these groups of bacteria. Wood (1953) noted that primary film forming bacteria tolerated a maximum of up to 250 p.p.m. of CuSO_4 . Of further interest is the fact that these organisms are also denitrifiers. They belonged to the genus *Pseudomonas*, *Flavobacterium* and *Achromobacter* (Sreenivasan, 1956).

Denitrifying bacteria.—Bacteria liberating gaseous nitrogen from nitrates appear to be quite abundant in tropical marine environments. They were quite often detected in 1 c.c. quantities of surface sea-water and sometimes in bottom water also. Chank flesh and pearl oysters invariably contained denitrifying bacteria even in 10^{-3} dilutions. They were capable of producing gas from the medium of Waksman *et al.* (1933a), as also from nitrate-peptone broth. These 'marine' denitrifiers were all monotrichous, non-fluorescent, gram negative rods belonging to the genus *Pseudomonas*. The denitrifying activities of similar bacteria have been studied in detail (Sreenivasan and Venkataraman, 1956). Utilization of amino acids by some of the denitrifiers has also been reported upon (Venkataraman and Sreenivasan, 1955). An account of chitinophilic denitrifiers is already given on page 360. It was noted that with only mineral salts, plus chitin and potassium nitrate, some of the chitinophilic bacteria liberated gas. So also with only ammonium sulphate, KNO_3 and glucose, gas was liberated by these, confirming our earlier observation that denitrification is possible in marine environments also. Besides these, some members of the genus *Bacterium* which digest alginic acid were also seen to be denitrifiers. The copper tolerant denitrifiers (page 360) may play some part in fouling by virtue of precipitation of CaCO_3 due to the denitrifying activities and thereby increasing the pH (ZoBell, 1946). The abundance and ubiquity of these denitrifiers and the poverty of nitrates in tropical waters explain the rôle of these in the depletion of nitrates.

Luminescent bacteria.—Quite a number of bacteria from sea-water were phosphorescent, but this property was lost on subculture. Bacteria isolated from pearl oyster showed a large number of highly luminous colonies. Wood (1953) also recorded the occurrence of such bacteria in Australian water.

Lactose fermenting bacteria.—Sea-water 10 miles off-shore (examined in three 'paars' at different periods) did not reveal the presence of coliforms. The chank flesh and pearl oysters contained some coliforms, the latter 17 per 100 ml. It is thought that they are adventitious and further work is necessary.

Sulphate reducing bacteria.—In the denitrification medium of Waksman *et al.* (1943), inoculated with bottom sand, at first nitrate reduction occurred with the liberation of nitrogen. Later, it was seen that there was blackening of the sediment accompanied by the smell of hydrogen sulphide, indicating the presence of sulphate reducing bacteria. Sea-water and other marine materials were inoculated into Van Delden's medium. Surface and bottom sea-water did not show the presence of sulphate reducers but the off-shore sand did. Chanks and pearl oysters showed positive sulphate reduction even in 1:1,000 dilution indicating the large numbers of sulphate reducing bacteria. These were found to be also denitrifiers. They were long, flexuous, motile rods. It was found difficult to obtain pure cultures of these by cultivation on the laboratory media. Better growth was, however, obtained in media with thioglycollate indicating the low-redox potential under which they grow.

TABLE

A description of agar

Strain No.	Cultural characteristics	Morphology	Broth	Potato
Sd.13	Orange yellow, circular, moist, undulate, smooth, butyrous, deep saucer-shaped depression and liquefaction. Fishy smell.	Rods, curved, C- & S-shaped, motile, long, thin, variable length, gram negative. No spores.	Turbidity, pellicle and thick ring.	Brown, warty growth.
Sd.14	Grey translucent, glistening, smooth, agar depressed.	Rods, short, ellipsoidal, non-motile, gram negative. No spores.	Turbidity and pellicle.	..
Sd.16	Saffron coloured, bright glistening, smooth, liquefying agar.	Rods, gram negative, long, curved, slender, non-motile, single and pairs. No spores.	Uniform turbidity. Orange red ring.	Scanty streaks.
Sd.17	Orange yellow, glistening, circular, smooth viscous. 'Crater' like depression and liquefaction of agar. Amine odour.	Gram negative, very long rods, curved motile, peritrichic. No spores.	Turbidity and pellicle. Broth turns brown.	..
Sd.18	Orange yellow, smooth, glistening, depression, formed and liquefied. Fishy odour.	Rods, straight, curved, etc., gram negative, motile, pleomorphic, peritrichous flagella.	Uniform turbidity.	Rose streaks.
SW.8	White, circular, entire depressed colony. Agar liquefied slowly.	Gram negative, single, pairs and chains, motile, monotrichous.	Turbidity and fragile white pellicle.	..
SW.9	White, circular, entire depressed, agar slowly liquefied. Foul amine smell.	Rods, motile, gram negative, coccoid, slightly curved, monotrichous.	Uniform turbidity. Fragile pellicle ray ring.	..

111

digesting bacteria

Carbohydrates, sugars	H ₂ S	Indole	Gelatin	Purple milk	Nitrate reduction	Growth in fresh-water media
Glucose, arabinose, sucrose, mannitol—acid; lactose—no change; starch hydrolysed.	Rapid liquefaction.	NC	Reduced to nitrate.	..
Starch hydrolysed; glucose, sucrose—acid; lactose—no change.	Rapid liquefaction.	NC	Reduced to nitrate.	..
Glucose, sucrose, mannitol acid, lactose—no change; starch hydrolysed.	Liquefied slowly.	NC	Reduced.	..
Glucose, sucrose—acid; lactose, mannitol—no change; starch hydrolysed.	Rapid liquefaction.	Alkaline peptonized.	Reduced.	..
Glucose, sucrose, mannitol—acid; lactose—no change; starch hydrolysed.	Rapid liquefaction.	Coagulated acid (slow).	Reduced.	..
Sugars—no change; starch hydrolysed.	Liquefied.	No.	Reduced.	..
Sugars—no change; starch hydrolysed.	Rapid liquefaction.	No.	Reduced.	..

TABLE IV
Alginic acid digesting bacteria

Culture No.	Cultural characteristics	Morphology	Broth	Sugars	Gelatin liquefaction	HS ₂ indole	Milk	Nitrate reduction	Fresh-water agar	Remarks
SW.10 SW.20	Gray translucent, flat, moist, circular; agar slant: gray white, moist, glistening, smooth; algin agar: circular convex, drop-like, entire, glistening; algin digested; agar softened.	Rods, gram negative, actively motile, peritrichous, single and pairs, 0.5-0.8 / 1.0-1.5 rounded ends. No spores.	Turbidity, pinkish, fragile pellicle and ring.	Glucose, lactose, mannite, arabinose—NC; starch hydrolysed.	Rapid		No			NH from 3 peptone.
SW.43	Gray translucent, flat adherent; agar slant: gray, smooth, glistening; algin plate: cleared the opacity.	Rods, actively motile, single and pairs, and short chains. gram negative. No spores.	Uniform turbidity	Glucose, sucrose, maltose—alkaline; lactose—NC; starch hydrolysed.			NC			
SW.44 SW.45	Circular, gray white, entire, smooth, glistening; agar slant: gray white, fliform algin plate: opacity cleared.	Rods, actively motile, gram negative, single and pairs. Medium sized. No spores.	Turbidity, membranous and tenacious pellicle.	Glucose, sucrose, maltose, lactose—no change; starch hydrolysed.	Rapid		NC	De-nitrified.		
SW.46	Circular, cream white, raised, entire, smooth; slant: cream yellow, butyrous, abundant, glistening, smooth; algin plate: raised circular, moist, digests algin, softened agar.	Rods, gram negative, non-motile, medium sized. Single and pairs. No spores.	Uniform turbidity	Glucose, sucrose, maltose—no change; starch hydrolysed.			Slowly alkaline.			

NC = No change.
— = Negative.

Urea-splitting bacteria.—Bacteria splitting urea and liberating ammonia were present in sea-water in 0.1 c.c. quantities in sand and in chanks. They were also recorded from the surface flora of sharks. The urea-splitting bacteria from chanks were studied in detail. Some of them produced ammonia from urea, but none appeared to be *proteus*. They were all gram negative, non-spore-forming rods, either motile or non-motile with polar or peritrichous flagella. The polar flagellated organism was identical with the denitrifying copper tolerant *Pseudomonas* described earlier.

Bacterial flora of sea-water.—Representative cultures of surface and bottom sea-water were subjected to detailed morphological, cultural and biochemical tests. As in our earlier studies (Venkataraman and Sreenivasan, 1954a, b) *Bacillus* was the single dominant group. Six of the *Bacillus* cultures were chromogenic, three being rose red. It is particularly interesting to note that these red *Bacillus* occurred quite often in sea-water and in fish in the west coast also. *Bacterium* and non-chromogenic *Pseudomonas* also occurred in some quantities. *Corynebacterium* and *Achromobacter* were found in lesser numbers. Other genera represented, but in insignificant numbers, are *Flavobacterium*, *Vibrio*, *Chromobacterium* and *Microbacterium*. Of the 35 cultures, nine had yellow chromogens and five red. Motility and gelatin liquefaction seem to be a normal property of marine bacteria: 26 cultures out of 35 were motile and 28 liquefied gelatin. As in our earlier studies, we noticed very few 'marine' bacteria fermenting sugars. However, all the cultures hydrolysed starch. Starch hydrolysis is fairly common (ZoBell, 1946; Wood, 1953) despite the fact that this form of carbohydrate does not occur in the sea. Two of the *Pseudomonas* produced indole and three H_2S , while one *Bacterium* produced H_2S . 'Fishy' and foul odours were produced on laboratory media by two *Pseudomonas* indicating the probable rôle in fish spoilage. Nitrate reduction was common among sea-water bacteria, 21 out of 35 reducing nitrates to nitrites and some of them even liberating nitrogen. Twenty per cent of cultures peptonized milk. Sixteen cultures are strictly 'marine' forms failing to grow in fresh-water media.

DISCUSSION

The productivity of the seas depends ultimately on the transformations of nitrogen and carbon compounds, mainly due to bacterial activities in the mud, water and the mud-water interface. The existence of a biochemically versatile flora assists in the mineralization of organic matter, decomposing even inert materials, such as lignin, chitin, cellulose, etc. As a result, mainly of the work of ZoBell and his associates, bacteria are also known to play a prominent rôle in petroleum genesis. A study of marine bacteria is also necessitated by their rôle in spoilage of fish with which we are more concerned.

The existence of a special bacterial flora as an ecological group is evident. The inhospitable nature of sea-water to terrestrial forms is clearly seen from the decrease of 'total' counts as well as coliform counts with progressive distance from the land. The presence in sea-water of agar digesting bacteria has been recorded by Waksman *et al.* (1933c), by ZoBell (1946) and by Wood (1953). Their presence in large numbers in the bottom sand and in the chanks will aid in the decomposition of this polysaccharide. The agar and algin digesting bacteria may also attack the algae themselves and decrease their content of these phycocolloids. The association of biochemically active groups of bacteria with chanks and pearl oysters is of benefit to these molluscs. It is indeed an example of 'mutualism'. The presence of cellulose digesting, chitin digesting, agar digesting bacteria, etc., perhaps assists in the digestion of these materials by those molluscs. Such symbiotic chitin digestion is also reported by Jenniaux (cited by Hackmann, 1954). Hungate's (1950) review that the cellulose digesting capacity of termites is due really to the presence of bacteria supports the above reports.

The occurrence of large numbers of denitrifiers on these animals need some explanation. The coral reef formation in tropics has been attributed to the activities of denitrifying bacteria, which precipitate CaCO_3 (Bavendamm, 1931; Drew, 1911, 1913). Probably the shell formation in these molluscs is aided by this process. The rôle of denitrifiers and sulphate reducers in calcium carbonate precipitation has been extensively and excellently reviewed by ZoBell (1946). Since sulphate reducers were also present in these molluscs, conditions for calcium carbonate precipitation are favourable, as the removal of acidic radicles like sulphates and nitrates leaves an alkaline medium necessary for calcium carbonate precipitation. At present, pearl formation in pearl oysters is attributed to the irritation caused by certain parasites. The possibility of bacteria initiating chemical 'irritation' and/or subsequently depositing CaCO_3 by a process of denitrification and sulphate reduction appears to be an attractive theory. In this connection Drew's experiments (1911) are worth citing---' . . . The addition to this culture of very fine particles of calcium sulphate, or of larger particles of sand, resulted in the aggregation around them of particles of calcium carbonate, forming a concentrically laminated concretion around a central nucleus. These were hard and of almost crystalline appearance Once this process of concretion has been initiated it appears to progress independently of the presence of particles which act as nuclei ' More of fundamental research is needed to elucidate the mechanism of pearl formation based on Drew's hypothesis cited above.

Bacteria tolerating such high concentrations as 750 to 1,000 p.p.m. of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ have not been reported so far. These bacteria isolated from chanks and pearl oysters are slimy and could probably form films on submerged materials. Their denitrifying faculty is further interesting in that this also assists in film formation (ZoBell, 1946). Thus, these 'copper' bacteria might assist fouling organisms by attacking the copper paint composition, by forming films, by forming the food of fouling organisms and in various other ways mentioned by ZoBell. The ubiquity and abundance of denitrifying bacteria are significant. They might certainly affect the nitrate content of sea-water and cause loss of nitrogen. Existence of bacteria producing gas from nitrates in the presence of chitin, algin, agar, etc., as enumerated in this paper, clearly indicates that denitrification can take place in the sea, despite the poverty of soluble form of organic matter. The part played by the mesophilic saprophytic bacteria in fish spoilage will be evident from the fact that the cultures were proteolytic and produced indole, H_2S and other offensive odours. The presence of large numbers of spore-forming bacteria is again a problem in heat processing of fish.

SUMMARY

The bacterial population of off-shore sea-water is sparse, but there were versatile physiological groups present to a small extent in sea-water and to a greater extent in the bottom sand and in benthic animals. The presence of these groups such as agar digesters, algin digesters, cellulose digesters, urea splitters, sulphate reducers, etc., brings about transformation of organic matter in the sea. By their symbiotic association with the chanks and other molluscs, it is thought that they aid in the digestion of chitin, cellulose and other stable polysaccharides. The presence of denitrifiers in sea-water and in association with the molluscs may bring about the precipitation of calcium carbonates in sea by removing the acid radicles, such as sulphates and nitrates, increasing alkalinity, etc. Whether a similar mechanism may bring about pearl formation needs investigation. The rôle of 'copper' bacteria in the fouling of submerged materials is discussed. Coliforms did not survive in sea-water beyond five miles from shore and the inflow of town sewage had no effect beyond to three miles from the shore. The bacterial flora of sea-water consists of *Bacillus*, *Pseudomonas*, *Bacterium*, *Corynebacterium*, *Achromobacter* and other genera of lesser importance.

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